

The changing of the old guard

The British government seems to have sensed that its policy on education could be an electoral liability, which is correct. But throwing printed money at the problem will not help.

Mr Kenneth Baker, the new Secretary of State for Education and Science in the British government, has with enthusiasm taken on an impossible task. After two years of bruising labour troubles in the schools, he has implicitly undertaken to restore the morale of the dwindling band of able teachers, to modernize the school curriculum and to produce results (better examination results). He has to do the same in higher education, somehow persuading academics and their institutions that the government's reduction of their budgets by more than 20 per cent in five years is in reality a measure of its high esteem for them. Harder still, he must persuade researchers (for the government has not taken this golden chance to split off the administration of research) that his predecessors have caused institutes to close, and cohorts of bright young people to find posts elsewhere, so that the remainder will have better pearls to look for. All this has to be done in less than eighteen months.

Mr Baker's enthusiasm is his strongest suit. It won him notable success when, as minister of information technology five years ago, he persuaded the British government and many of its electors that the time had come to "cable Britain", putting umpteen channels of cable television into every home. He had fortunately been promoted from that post by the time it became apparent that he had not persuaded that section of the community with funds sufficient to launch commercial cable that there could be no better place for their money. It would nevertheless be a great misfortune if the transformation now planned for British education follows the pattern of the cable revolution that never happened. Education is too important.

The most chilling sentence in last week's letter from the University Grants Committee to its constituents is the statement that there is now no prospect of being able to increase the proportion of university students following courses in the government's special engineering and technology programme by more than 14 per cent over 1984–85 by the end of the decade because of "early specialization and staffing" in the schools. Similar constraints apply elsewhere across the educational spectrum, and governments are not entirely to blame. British universities have been chiefly influential in requiring that school-leavers should have so thoroughly rehearsed at school what they will learn at university that students are at once bored (because they know it all already) and ill-educated (because they know nothing else). The universities have now changed their tune.

So how should Mr Baker best blend his endearing personal enthusiasm with the long-term character of the problem of education and research? Here are a few maxims he might follow. Most parts of his new intellectual empire have been through a long period of change, which has accelerated in the past few years. The schools had hardly survived the intense curriculum development of the 1960s when they were required to change again, for largely social reasons. Higher education began that period by being expected to grow quickly and was then asked to stand still until the time came to contract. The same has happened in research. People are sick and tired of it. A moratorium on organizational revolution would be a blessing.

An end to upheaval does not however require stagnation. Mr

Baker would do himself and his constituents a power of good if he allowed that, within a framework of stability, it would be in the public interest if people or institutions who wish to change were allowed to do so. There would be some schools keen on taking up new bits and pieces of curriculum, even some universities anxious to break out in new directions, not to mention a small army of academic researchers who would eagerly do new things. Inspection might even discover that the Royal Greenwich Observatory, about to be moved for largely administrative reasons, might usefully be left where it is on the understanding that its professional astronomers should compete for research grants with their colleagues in the universities.

To whom should even an enthusiastic new minister turn for advice on matters like these? The trouble with the British enterprise in education and science is that its managers have been tainted by the failures of the past. All honourable men and women, they are the same who have failed to defend their institutions from internal weaknesses and from the malevolence of Mr Baker's predecessors. They have lost the allegiance of their younger and more creative colleagues. Mr Baker, if his intentions are serious, had better get out and about, off the beaten tracks of the corridors of administrative tidiness. While on his journeys, he might make the speech or two suggesting that he is someone who knows what the enterprise is for. □

What yellow peril?

The British government has said it has not found mycotoxins in samples of yellow rain.

THE notion that the people of Laos and Cambodia were attacked by their Vietnamese neighbours by means of a chemical toxin sprayed from aircraft had become a bit of a joke even before Dr Matthew Meselson turned it into pastiche by remarking that most of what appears to be yellow rain consists of the excrement of bees. The allegation was first made by Mr Alexander Haig during his brief spell as US Secretary of State, but on the flimsiest of evidence. Trichothecene toxins, produced naturally by *Fusarium* fungi, are undeniably lethal in appropriate circumstances, but less quickly so than cheaper modern chemical warfare agents.

As time has passed, the case has been less easy to sustain. People's capacity to analyse for trichothecenes is one of the marvels of mass-spectrographic assay. The fact that Britain's Porton laboratory has looked for trichothecenes in yellow rain and failed to find them does not imply that all samples of yellow rain are free from mycotoxins, or that no yellow rain has ever been so contaminated deliberately. But it does give the lie to the notion that all yellow rain is lethal, which is not surprising since most of it is known and admitted to be the excrement of bees. So now there are two choices; either the State Department must acknowledge that the Vietnamese were even more devilishly clever than had been supposed in concealing their use of an improbable chemical weapon behind the cloak of a previously ill-recognized biological phenomenon, or the State Department should allow that it may have been mistaken. □



Genetic regulations

US to settle dispute over agency jurisdiction

Washington

AFTER months of inter-agency wrangling, the new federal government policy statement on biotechnology research is expected to be published in the *Federal Register* by the end of the month. Its supporters believe it will make clear the complex maze of regulatory jurisdictions among federal agencies. But even its authors acknowledge that the new document, which is two-and-a-half inches thick, will be a challenge to general understanding.

The framework is the next step in the process begun in December 1984 to coordinate government policy regulating biotechnology (see *Nature* 313, 85; 1985). Since last November, when an index of relevant statutes appeared in the *Federal Register*, the Domestic Policy Council (DPC) Working Group on Biotechnology has been conducting an intensive effort to arrive at an inter-agency consensus on how those statutes are to be applied.

"The goal was to get coordination and consistency", says David T. Kingsbury, National Science Foundation (NSF) Assistant Director for Biological, Behavioral and Social Sciences and chairman of the DPC working group. The effort attempts to get away from a process approach to regulation, and move toward a product oriented approach.

The DPC working group felt that

placing all products created by recombinant DNA techniques under the same regulatory umbrella was misguided. As an example, the pseudorabies vaccine developed by Saul Kit at Baylor College of Medicine uses a modified virus missing a part of its genome. Because it incorporates no new genetic material, it may reasonably be treated just like any other altered vaccine. The working group takes the approach that it is only when genetic material from organisms of different genera are combined that special attention needs to be paid to the potential hazards of a new organism.

The new document, the Coordinated Framework for Regulation of Biotechnology, contains policy statements by all the major regulatory agencies involved in biotechnology. Because of the "mosaic" of statutes covering biotechnology issues, "the potential for confusion is enormous," says Kingsbury. To ameliorate this problem, the new framework proposes two new definitions as a basis for coordinating new regulations.

The Biotechnology Science Coordinating Committee (BSCC), made up of senior policy officials of agencies involved in biotechnology research and products, took responsibility for coming up with the definitions. An "intergeneric organism (new organism)" is one formed by "delib-

erate combination of genetic material from sources in different genera". A "pathogen" is defined to include microorganisms that "belong to a pathogenic species or that contain genetic material from source organisms that are pathogenic." Specifically excluded from both these definitions are organisms that are "well characterized and contain only non-coding regulatory regions." BSCC is considering expanding these exemptions, as well as defining more formally an environmental release.

A key accomplishment of the new framework is to sort out the overlapping jurisdictions of the various federal agencies involved in biotechnology regulations. The framework attempts where possible to identify the single agency having responsibility for a single product, but where overlapping jurisdictions arise, the lead agency is identified. Kingsbury, as chairman of BSCC and DPC working group, will be the *de facto* arbiter of disputes over jurisdiction.

While sorting out overlapping jurisdictions between agencies was a major accomplishment for the DPC working group, jurisdictional issues also cropped up within the agencies. At the US Department of Agriculture (USDA), disputes arose between the science and education division and the marketing and inspection services over review issues.

One upshot of the disputes was the creation of an Office of Agricultural Biotechnology to keep track of all items either reviewed for licence or proposed as research. In addition, the Agriculture Recombinant DNA Committee (ARC) is being disbanded; an Agricultural Biotechnology Recombinant DNA Advisory Committee will now assume responsibility for review of all research projects.

Although the new framework represents a step forward in coordinating federal policy, some worry that the regulatory system is still chaotic. Quick to acknowledge the magnitude of the task Sue Tolin, co-chair of the USDA ARC, is unconvinced that the new framework will sort out all the regulatory tangles now confronting biotechnology research firms. She points to a discouraging catch-22 situation; to prove organisms are safe, they must be tested, but before they can be tested they have to be proven safe.

Meanwhile, the first attempts at actually field-testing a genetically engineered organism are still running into problems. The Environmental Protection Agency declined to issue an experimental use permit to Monsanto to test two strains of *Pseudomonas fluorescens* containing a gene insertion of a toxic gene from the microbial insecticide *Bacillus thuringiensis*. The agency wants to see additional data on the organisms' environmental impact and short-term pathogenicity.

Joseph Palca

Chernobyl

British embassies turn monitors

AN attempt at comprehensive monitoring of the radioactive fallout from the Soviet nuclear accident at Chernobyl has been carried out by British embassies in European capitals bordering the Soviet Union. A statement put out last week by the National Radiological Protection Board and by the Foreign and Commonwealth Office (FCO) suggests that contamination is greatest in north-east Poland, Czechoslovakia and Hungary.

The FCO statement says, however, that the contamination of local foodstuffs is nowhere high enough to suggest that travellers to those parts of Europe would receive "more than a fraction" of the annual dose limit for members of the general population. The statement adds that travel to Kiev and the western Ukraine, and to Minsk and Byelorussia, should be avoided if possible, but that simple precautions (such as the avoidance of fresh milk and leaf vegetables) would be sufficient elsewhere in the Soviet Union and

Eastern Europe.

The use of the network of British embassies as a means of monitoring fallout from Chernobyl was made possible by the supply of simple equipment immediately after the accident. According to figures accompanying the statement, the dose rate at Kiev, south of Chernobyl, had fallen from 30 to 2 microsieverts an hour between the period immediately after the accident and 14 May. Elsewhere in Eastern Europe, the highest dose rates recorded by British embassies were in north-east Poland (4.5 microsieverts an hour) and Romania (3.6 microsieverts an hour), but these had fallen to 0.8 and 0.9 microsieverts an hour by 14 May.

By comparison, the estimated radiation dose rate due to natural causes (cosmic rays, radon and internalized natural radioactive species) is generally taken to be 2 millisieverts a year at normal altitude and in mid-latitude, roughly the equivalent of 0.5 microsieverts an hour. □

British universities

Further contraction ahead?

BRITISH universities were last week divided in their estimation of what the future holds. The replacement as Secretary of State for Education and Science of Sir Keith Joseph, who has retired from the government, by Mr Kenneth Baker, previously Secretary of State for the Environment, was generally regarded as a sign that the government may change course on the continued contraction of the university system. But the letter to universities from the University Grants Committee (UGC) has confirmed others in the belief that deprivation will continue.

Mr Maurice Shock, chairman of the Committee of Vice-Chancellors and Principals (CVCP) and vice-chancellor of the University of Leicester, in a fierce statement after the publication of the UGC letter, said that the present gap between the universities' needs and the total recurrent grant from UGC amounts to 4 per cent of the total budget, not merely the 2 per cent of annual income estimated by UGC. That estimate, he said, had been made in "far-off, happy, dreaming days". Now, "you can forget about 2 per cent".

The worsening of the immediate financial prospects for the British universities has come about for several reasons, most of them outside the control of the universities themselves. One is the unexpected increase in the amounts universities have to pay as rates (local authority taxes), which have risen for the university system as a whole by 13 per cent as a consequence of government policies on central subventions for local government.

The cost in the present financial year amounts to £15 million for all the universities, roughly 1 per cent of their total cost. Shock said last week that if the British government wished to give an earnest of its intention to deal more generously with the universities (see *Nature* 321, 370; 1986), as promised by Sir Keith Joseph in his pre-retirement statement to the House of Commons, it should make good this shortfall.

Academic salaries (which account for 70 per cent of academic costs) have become another issue between the universities and the government, chiefly because the university budget for the coming year has been drawn up on the assumption that salaries would increase only in line with inflation, itself supposed to be 4 per cent a year, while the government has just concluded a pay settlement with its civil servants that will give them more than 6 per cent a year.

CVCP's pessimism is borne out by the details given in the UGC letter of subventions for universities in the year 1986-87 (which begins on 1 August). By that time, UGC says, it will have become

apparent that the university system will have fallen back during the year, relative to inflation, by about 3.5 per cent and that in future the shortfall will exceed 2 per cent in each year.

This year's letter to universities is of particular interest in that it is the first in which UGC has based its allocations on a deliberate assessment of the quality of individual institutions both in research and in quality of teaching. Over the succeeding three years, the proportion of the total UGC budget (£1,213 million in 1986-87 after deduction of rates and other special amounts) likely to be redistributed between institutions to allow for differences in research output is expected to amount to 15 per cent. But in this first year of the new regime, the consequences of these judgements have been limited to variations of between 1 and 3 per cent in the grant to individual universities.

The result is that it is not possible to tell from the figures published last week which

universities are likely to prosper in the coming years and which will be in danger of extinction. Two newer universities (Warwick and Bath) do best out of the allocation, with grant increases between this year and next of 4.0 per cent and 3.6 per cent respectively. Oxbridge marks time (the Cambridge budget goes up by 0.7 per cent between this year and next, that of Oxford is unchanged). Universities such as Essex and Hull, the business schools and the Welsh university colleges will have their budgets cut by 0.5 per cent between this year and next, but a full explanation will be apparent only when individual letters are sent to universities early this week.

The prospects for an improvement of the situation turn entirely on a proposal in Sir Keith Joseph's final statement that there should be talks between the universities and the government on the financial situation, and the promise that extra funds would be made available to universities able to demonstrate that they had met certain objectives, not yet clearly defined. Will Mr Baker be able to put flesh on the bare bones of this promise? □

Yellow rain

British analyses find no toxin

THE British government last week disclosed that attempts to find traces of mycotoxins in samples of yellow rain from South-East Asia have yielded negative results. In reply to a question from Mr Leo Abse, Member of Parliament for Pontypool, on 19 May, the Ministry of Defence acknowledged what has been widely rumoured for some time, that the British work so far has failed to confirm reports from US laboratories of the presence of trichothecene mycotoxins collected from Laos and Cambodia.

It is understood that the British investigations have been carried out at the Chemical Defence Research Establishment of the British defence ministry at Porton in Wiltshire. Samples of yellow rain collected from South-East Asia were supplied by the US authorities. The British laboratory was involved in a collaborative study of the composition of yellow rain after the first allegations in 1981 by the US State Department that yellow rain was an agent of chemical warfare were followed by reports of mycotoxins, notably by Dr J. Mirocha of the University of Minnesota.

The British government said last week that the Porton laboratory has analysed a number of "environmental and biomedical samples" from South-East Asia "to see whether trichothecene mycotoxins could be detected". Noting that the absence of positive results is "not necessarily incompatible" with positive findings elsewhere from different samples, the state-

ment nevertheless says that "we are confident" that the techniques used at Porton would have detected mycotoxins had they been present in the "alleged chemical warfare" samples available.

The statement by Mr John Stanley, Minister of State for the Armed Forces at the Ministry of Defence, went on to say that, in spite of the negative findings in the analysis is at Porton, the British government believes that "chemical warfare attacks probably did take place in South-East Asia" on the basis of "epidemiological evidence". He added that the British government is unable to say what agent may have been used in those attacks, or who may have supplied it.

In reply to one of the points raised by Mr Abse, the British government said its investigations "neither support nor contradict" the suggestions made chiefly by Dr Matthew Meselson of Harvard University that drops of yellow rain of the kind reported from South-East Asia are indeed the faeces of bees.

The British government's statement is important because of repeated suggestions from the United States that the British measurements confirm the positive findings in some US laboratories. The "epidemiological evidence" referred to in the government statement presumably refers to reports of mycotoxins in the blood of members of the Hmong population of Laos and Cambodia who have reported being attacked by chemical agents in the form of yellow rain. □

US research control

Professional societies still at sea

Washington

DESPITE the attention given in recent years to restrictions imposed by the US Department of Defense (DoD) on foreigners' access to technical meetings, professional societies still differ widely in their policies on the sponsorship of closed or restricted sessions.

According to the committee on scientific freedom and responsibility of the American Association for the Advancement of Science (AAAS), many societies apparently deal with DoD in an *ad hoc* fashion. Fewer than half of 45 technical societies affiliated to AAAS that responded to a questionnaire have adopted policies dealing with the question, but those that have generally favour prohibiting restricted-access sessions.

Government policy on open communication has been subject to several revisions in recent years. Recently, controversy has focused not so much on security classification as on export controls used to prevent access by foreigners to unclassified research results. Last October, the White House issued a security directive stating that the administration intended to use only security classification to control access to fundamental research data, to the general approval of the research community. But the imposition of export controls on meeting sessions for limiting access to applied research remains contentious.

Several important scientific societies, including the Institute of Electrical and Electronic Engineers (IEEE), have said they will not sponsor such sessions under any circumstances. Foreigners sometimes voice the suspicion that such controls are imposed more to give US industries a competitive advantage than to protect national security.

The 45 affiliated societies responding to the questionnaire were selected for their interest in subjects likely to be militarily important. Forty per cent had adopted policies on restricted sessions, almost half of them within the past five years; two-thirds of those with explicit policies restricted or closed meetings. The report concludes that if more societies were to adopt formal policies, these would probably prohibit closed sessions.

A total of four societies had actually experienced "problems" with access by foreign participants in the past three years, one of them more than five times. A separate survey of 24 technical societies not affiliated with AAAS drew only 8 responses; 5 of these societies had experienced problems with access by foreigners to meetings. The AAAS report suggests that the response rate of the non-affiliated societies was poor because the

subject is politically sensitive.

DoD's most recent proposal on the subject was in a *Federal Register* notice on 12 February (p.5210). DoD responded to professional societies' concern by undertaking to complete security reviews of the research it sponsored in not more than 30 days, except in "rare and exceptional" cases. DoD also informally undertook in meetings organized by IEEE to ensure that security reviews would be conducted by scientifically qualified personnel, rather than by security inspectors who would "see one key word on a page and classify the whole thing", according to one IEEE negotiator.

The February statement did, however, stir already muddy waters by appearing to contradict the October White House

directive on fundamental research, declaring that DoD policy is to allow the publication of unclassified fundamental research only "to the maximum extent possible". But the October security directive definitely still stands, according to Leo Young, director of research and laboratory management in the Pentagon's office of research and advanced technology.

Young said his office had erred in not making this clear in the February statement, but, he said, the great majority of responses from societies to the proposed policy had been favourable. Young said he hoped societies could avoid holding export controlled sessions, but that some, such as the Society of Photo-optical Instrumentation Engineers, were likely always to be in sensitive areas, and that it was up to them whether or not to restrict sessions at their meetings by the use of the export controls.

Tim Beardsley

Ethiopia

Brief respite from famine

Addis Ababa

IT is now raining in Ethiopia, and much of the country has turned green as a variety of arable crops and pasture have begun to grow again. But this has not chased away the general belief that Africa is a continent in crisis. Dr Mostafa Tolba, executive director of the United Nations Environment Programme (UNEP) based in Nairobi, Kenya, is planning to use the special meeting of the United Nations in New York from 27 to 31 May as a chance to emphasize that the current problems of Africa stem from the underlying deterioration of the African environment.

In Ethiopia, the drought and subsequent famine of 1984–85 has been the focus of attention, but the underlying question is why the ill-effects of the drought were so serious. There were at least eight serious droughts earlier this century, but none of them had such disastrous consequences.

The rapidly expanding population concentrated in the highlands of the east is one of the most conspicuous problems. The traditional pattern of settlement is that of a regular distribution of homesteads across the cultivated areas, but the density is now so high that the marginal land on the steep slopes of the mountains and precipitous river gorges has also been cleared and settled.

One consequence is that the loss of traditionally dense forest to firewood and building lumber has been enormous. In twenty years, the proportion of wooded land has fallen from 16 per cent to about 3 per cent. The result is increased erosion of the soil whose capacity for water retention is itself reduced. At the same time, UNEP officials say, the productivity and drought

resistance of the land is falling, which puts pressure on subsistence farming especially when the preferred crop (teff) is lower yielding, less drought-resistant and requires more work than near-equivalents such as millet.

The vulnerability of the land to drought is made worse by poor communications and the preoccupation of the government with rebellions in at least three regions. Referred to as "structural problems" by Commissioner Tadesse of the Government Relief and Rehabilitation Commission, their significance is not underestimated. But the continuing internal conflicts make the outcome for the next drought look bleak.

Similar environmental problems in other African countries led governments to attend the first African Environment Conference, organized by UNEP, the Organization for African Unity and the Economic Commission on Africa, held last December. The outcome has been an increased awareness of the seriousness of environmental deterioration and a strong commitment to cooperation in obtaining the best possible assessment of problems such as desertification and water use to provide a reliable basis for appropriate development.

Although the piles of wheelbarrows now accumulating in the distribution centre of the aid agency, Worldvision, in Wallo region attest to the changing emphasis as the current crisis passes, any major reversal in the trend of environmental deterioration will require an awareness at all levels of the importance of the problems and the enormous collaborative effort required to tackle them.

Nigel Williams

Japan

Another academic city planned

Tokyo

PLANS are being laid for a large-scale academic city in the Kansai area of Japan. It is hoped that the city will be approved as the region's next giant construction project following the completion of the new international airport at Osaka. Its proponents do not see it as a rival to Tsukuba Science City near Tokyo; it will aim more at basic research and cultural activities than Tsukuba, which is dominated by government research laboratories.

Two thousand five hundred hectares of land have been set aside for the city where the prefectures of Kyoto, Osaka and Nara meet. The location is within 30 km of Kansai's major cities of Kyoto, Osaka and Kobe with their large state universities, and the ancient capital of Nara.

The region also contains the headquarters of a number of Japan's high-technology companies, including several of those involved in research in biotechnology and new materials, and many are proud of being "Kansai" as against "Kanto" (Tokyo zone) companies. Contributions from companies and relocation of their research institutes could very significantly help construction of the new academic city.

The nucleus of the city is at present contained in the "International Institute for Advanced Studies", the only part of the city that has passed from a dream to a reality. The institute was set up, in name at least, in 1984 on the initiative of two former presidents of Kyoto University, Professor Azuma Okuda and Professor Michio Okamoto, a member of the Prime Minister's Council for Science and Technology and head of the Extraordinary Reform Council for Educational Reform.

Since then, the institute has successfully passed through its "phase one": raising a capital of 133 million yen (\$830,000), largely from Kyoto-based companies, to establish a foundation and open an administrative office. Now it is well into "stage two" in which 3,000 million yen (\$18.7 million) is being raised to endow the foundation and to allow construction work to begin. With luck the institute will be built and running in five years' time. It was conceived after the study of successful advanced studies institutes elsewhere, notably those at Aspen and Princeton and the Rockefeller Institute, as well as other unique research institutions, including the Research Triangle Park of North Carolina and the Cavendish Laboratory (*Nature's* issues of 1874 providing a record of its establishment).

As in other advanced studies institutes, the aim will be to invite "the foremost scholars of the world" to come and work there for periods of a few years at a time.

Early plans for the institute suggest it will provide an exceedingly pleasant environment: Japanese-style buildings containing guest houses, seminar rooms and dining halls are to be laid out in parkland and connected by the covered corridors found in temples and traditional houses.

The Advanced Studies Institute has in fact already begun activities with the sponsorship of international seminars in Kyoto on artificial intelligence and biosciences. More seminars, centred on scholars invited from abroad, will take place until the institute can assume its full functions.

Several other new facilities may serve as kernels for the academic city. Among them are a new branch of the National Library; a new graduate university, linked to the joint-use university research facilities like those at Okazaki; and a new laboratory

for basic research in electrocommunications to be run jointly by the Ministry of Posts and Telecommunications and the newly privatized Nippon Telegraph and Telephone Corporation. These are, at least, possibilities that are being pursued; whether they can be turned into realities remains to be seen.

Those supporting plans for the new academic city believe it will be complete within 15 years. But there remain differences of opinion about its final form. There are some in the universities nearby who see it as a chance to relocate existing departments requiring expansion or renewal, or to bring together related institutes from different universities in the areas.

Others see the creation of a new academic city as a chance to break free from restrictions in the Japanese university system and to create a new style of institute, where deficiencies perceived in Japan's basic research contribution can be rectified.

Alun Anderson

Synchrotron sources

Stanford versus Argonne?

Washington

WITH pressure on the big science budget likely to increase in coming years, gentlemanly competition seems imminent over a proposal by the Stanford Synchrotron Radiation Laboratory (SSRL) to expand material research facilities at one of its electron storage rings. Recent experiments with the ring have been so successful that adding more beam lines might reduce the need for a future 6 GeV synchrotron coveted by Argonne National Laboratory.

The PEP (Positron Electron Project) ring at Stanford, generally agreed to have been something of a disappointment, was originally used for high-energy physics experiments, but with the impending completion of the Stanford Linear Collider (SLC), particle physicists have rather lost interest in it. In its heyday, PEP had five beam ports for high-energy physics experiments, but this figure is now down to one.

PEP might, however, be ideal for materials science measurements with synchrotron radiation if suitable beam ports and insertion devices were installed. For this purpose, the ring has to be run in a low-emittance mode. Although not designed for such a purpose, the machine excelled itself when low trials were conducted in March this year, reaching emittances close to those intended for the planned 6 GeV machine. It can reach energies of up to 8 GeV at low emittance, and if it were turned over exclusively for synchrotron radiation studies, 20 or more beam lines could be installed, according to Arthur Beinenstock, director of SSRL.

The Department of Energy (DoE) is indeed this week reviewing a proposal from SSRL to add three more beam lines to PEP, bringing the total to five. The proposal observes coyly that the existing beam lines have demonstrated "an emittance and dynamic aperture comparable to other potential storage rings presently being evaluated". The ring's long straight sections make it ideal for adding insertion devices such as undulators and wigglers.

The proposal to build a 6 GeV synchrotron radiation facility at Argonne by 1992 at an estimated cost of \$300 million has not yet received the official go-ahead from DoE, although its 1987 federal budget request includes some funds for preconstruction research and development. For the time being, extending the radiation facilities at PEP is being considered just as a means of paving the way for the dedicated 6 GeV machine, according to Lou Ianniello of DoE's office of basic energy sciences.

But Don Stevens, deputy associate director, adds that "it's a valid question" to ask whether an expanded PEP might not now be able to do all that the 6 GeV machine could do and one that "will have to be addressed".

Others are not so impressed. Ken Kliewer, who heads the 6 GeV project at Argonne, says that a dedicated above-ground machine could have up to 60 beam lines, and "Stanford can't match that". Kliewer thinks it unlikely that DoE will approve adding 20 or even 10 beam lines at PEP, in part because of the expense of necessary underground tunnelling.

Tim Beardsley

French reactors

Near-catastrophe at Le Bugey

A FRENCH pressurized water reactor (PWR) at Le Bugey on the Rhône river, not far from Geneva, came very close to a serious nuclear accident in 1984 after the failure of a single electrical component, a rectifier, it was claimed last week in the French satirical weekly, *Le Canard Enchaîné*. The French national utility, Electricité de France (EDF), and the atomic energy commission, the Commissariat à l'Energie Atomique (CEA), immediately held press conferences and admitted that the satirists were right.

There had been no cover-up, insisted Pierre Tanguy, head of safety at EDF, in a telephone call from Paris; a press release had been issued at the time, but "there had been no Chernobyl incident" to sensitize the media to nuclear issues.

What took place on the night of 14 April 1984 on the five-reactor site at Le Bugey, however, showed how a "very minimal failure" in a control circuit can give rise to a major incident, said Tanguy.

The event began with the failure of the rectifier supplying electricity to one of the two separate 48-V direct-current control circuits of the 900-MW reactor, which was on full power at the time. Instantly, a battery pack switched in to maintain the 48-V supply, and a warning light began to flash at the operators in the control room.

Unfortunately, the operators ignored the light (if they had not they could simply have switched in an auxiliary rectifier). According to Tanguy, the same light served three warning systems (to save space on the control panel). The installation on one of these other circuits had proved faulty a few days before, and the light had been flashing intermittently. It was marked down for repair, and ignored when it began correctly to signal the rectifier failure.

What then happened was something which had been completely ignored in the engineering risk analysis for the PWR. The emergency battery now operating the control system began to run down. Instead of falling precipitously to zero, as assumed in the "all or nothing" risk analysis, the voltage in the control circuit steadily slipped down from its nominal 48-V to 30-V over a period of three hours. In response, a number of circuit breakers began to trip out in an unpredictable fashion until finally the system, with the reactor still at full power, disconnected itself from the grid.

The reactor was now at full power with no external energy being drawn from the system to cool it. An automatic "scram" system then correctly threw in the control rods, which absorbed neutrons and shut off the nuclear reaction. However a re-

actor in this condition is still producing a great deal of heat — 300 MW in this case. An emergency system is then supposed to switch in a diesel generator to provide emergency core cooling (otherwise the primary coolant would boil and vent within a few hours). But the first generator failed to switch on because of the loss of the first control circuit. Luckily the only back-up generator in the system then did switch in, averting a serious accident.

A report of the whole sequence of events was distributed through the Organisation for Economic Cooperation and Development (OECD) "incident reporting system", said Tanguy, and PWR constructors and operators got together to consider the implications of the incident. "It drew everyone's attention to the possibility of a complete blackout of a nuclear plant", and was probably "the most significant event in relation to safety in a PWR" that has occurred.

The Three Mile Island incident, where operator error led to the venting of primary coolant and the ultimate collapse of the core of the reactor, realerted utilities to the well-known dangers of "loss of coolant accidents", or LOCAs; and so much attention has been paid to those that the danger is now minimized, according to Tanguy. But the Le Bugey incident shows that a whole new class of possible events had been ignored — those where electrical systems fail gradually. It shows that "risk analysis must not only take into account a yes or no, working or not working, for each item in the reactor, but the possibility of working with a slightly degraded system".

Such analysis, involving continuous in place of discrete variables, is very difficult. The French reaction had been to undertake such studies where possible, but also to introduce voltage monitors on critical circuits, to make sure that electrical values stayed within the ranges assumed in the discrete risk analysis.

Other measures taken since Le Bugey have been to introduce a single gas turbine generator that can be started up "within minutes" on each reactor sites, to maintain power to control and safety circuits, and to lay physical cable connections to allow a rapid emergency interconnection of generators between one reactor and another on each site if this proved essential. The critical "three warning system" lights in the control room have also been separated into multiple lights and circuits.

The occurrence of the unpredicted Le Bugey and Three Mile Island incidents had led to a lower probability of such events occurring again Tanguy's view is that accidents were inevitable, but with experience they become less and less likely. Which is why it is essential to learn every detail about what happened at Chernobyl.

Robert Walgate

Biotechnology companies

T cells' turn to ride high

Washington

ONE of the more unusual new biotechnology companies, T Cell Sciences of Boston, made its first public stock offering last week, raising \$12.6 million to continue its development of diagnostics and therapeutic agents focusing on T-cell receptors, in particular the T-cell antigen receptor. The company, founded two years ago by Dr Patrick Kung, has sewn up collaborative research agreements with many of the most prominent US research groups working on T-cell receptors, a remarkable feat given that basic understanding of the subject has been achieved only in the past two years.

The company, which now has about 30 employees, has centred its efforts on developing monoclonal antibody-based products that exploit the correlation of T-cell receptors and disease states. Its one current product (for research only) is ACT-T-SET, which is intended to distinguish chronic and acute immune disorders by assessing immune system activation through expression of very late antigen and interleukin-2 receptor.

Diseases that might be diagnosed through characterization of T-cell struc-

tures include various leukaemias, autoimmune diseases such as rheumatoid arthritis and systemic lupus and, perhaps, some cancers and acquired immune deficiency syndrome. Monitoring of the immune system for transplant rejection is another area where the company hopes to develop products. Later still, therapeutic products may be devised that would activate and control the immune system.

T Cell Sciences has a \$4.7 million research contract with Syntex Corp, and has licensing agreements with Ontario Cancer Institute, California Institute of Technology, Dana-Farber Cancer Institute and Stanford University, among others. Jim McCamant, editor of *Medical Technology Stock Letter*, comments that T Cell Sciences seems to be typical of a trend in which biotechnology companies are increasingly basing themselves on more speculative ideas and basic science, as the obvious technologies have already been adopted by the larger companies. Nevertheless, according to McCamant, the degree to which T Cell Sciences has established collaborations with major research groups makes it unusual, and means "you have to take it seriously". **Tim Beardsley**

East European nuclear power

Bloc countries think again

THE Soviet Union's Comecon partners have responded to the Chernobyl disaster, predictably, by endorsing the Soviet line that Western commentators have overdramatized the danger of nuclear power for political motives. While, as a result of the accident, Finland has cancelled its fifth and Yugoslavia its second nuclear power plant, the Comecon allies have spoken up firmly in favour of nuclear power. Czechoslovakia, Cuba and East Germany have been particularly vocal.

Within the bloc, the only indirect official criticism of Soviet and Comecon nuclear policy came from Hungary's chief spokesman on Foreign Affairs, Mr Matyas Szueres, who publicly urged the "inalienable right" of all people to knowledge in all matters which concern them, and the duty of all concerned with science and technology to respect public fears and concern, whether or not those fears were justified, a statement widely interpreted as a hit at Soviet reticence on the consequences of Chernobyl.

Poland, which caught the first phase of fallout from Chernobyl and which is at present building its first nuclear power station at Zarnowiec, is a special case. Clearly, after implementing a mass distribution of iodine pills to under-16s, ordering cows to be kept indoors and destroying contaminated vegetables, the authorities could hardly proclaim that nuclear power was without any hazard. Official statements have therefore urged that the Chernobyl accident will make the Polish authorities "additionally aware of improvements to all safety measures", and that in any case, the Zarnowiec reactor will be of the pressurized water type, quite different from that at Chernobyl, and will have, unlike reactors in the Soviet Union, a containment building. These assurances have clearly not been sufficient. Three thousand citizens of Bialystok, close to the eastern frontier of Poland, the area most affected by the initial fallout, have signed a petition to the Sejm (parliament) asking for the Zarnowiec plant to be cancelled, and five scientists working in nuclear physics and related fields have sent an open letter to

the Minister of Energy, General Czeslaw Piotrowski, calling for more stringent safety tests at Chernobyl. At his regular press conference last week, government press spokesman Jerzy Urban said that he knew nothing of these documents.

Dissident opinion in Eastern Europe has been somewhat divided on the issue of Chernobyl. In Poland, some small anti-nuclear protests took place on 1 May and 3 May (Constitution Day), and underground Solidarity newsletters have attacked the reluctance of the Soviet and Polish governments to publish sufficient details. (The Polish government, in fact, has been fairly open with contamination statistics, at least at the international level.) In Czechoslovakia, the Charter-77 movement likewise put out a statement urging the public's right to be fully

informed. But in Hungary, the "green" dissidents of the Danube Circle declined to comment, noting only that the hazard of accidents at nuclear power stations was in their opinion less of a threat to the environment than the Gabčíkovo-Nagymaros hydroelectric project which would disrupt the ecological balance even when it was working properly. And in the Soviet Union itself, although there was some attempt to organize an anti-nuclear protest last week in Moscow, no less a person than Academician Andrei Sakharov has backed up the official Soviet line. Sakharov, whose place of enforced residence, Gor'kii, will be the site of the first joint power/district heating nuclear plant in the Soviet Union, said last week, in a message to his relatives in America, that he still viewed nuclear power as inherently the best option. Western reporting in Chernobyl, he said, echoing the official Soviet statements, has been considerably exaggerated. **Vera Rich**

Chernobyl**Improvising against radiation**

THE Chernobyl clean-up has demanded "non-standard" and "unexpected" solutions, Academician Evgenii Velikhov, Vice-President of the Soviet Academy of Sciences, told the Moscow journal *Ogonek* last week. But what are these solutions? The accounts in the Soviet press, which concentrate more on the heroism involved than the technical details, give the impression of a series of improvisations worked out after the accident.

According to Ivan Silaev, one of the deputy prime ministers of the Soviet Union, the cost of a Swedish decontamination fluid at \$18.5 a kilogram was so high that a rush effort by Soviet scientists was necessary. By 14 May, production had reached 30 tonnes a day, allowing 300,000 m² of contaminated area a day to be coated with the material. Silaev also said, in an interview in *Izvestia*, that exposed surfaces of buildings were being coated with sodium silicate solution to prevent rainwater carrying contamination into the groundwater and to the river Pripyat.

Operations around the damaged reactor itself have been organized in typical Soviet style, with planned targets and campaigns to beat them. The miners drilling under the reactor have a target of five reinforcement rings installed per shift (giving a nominal rate of progress of 14 to 15 m a day, twice that of normal mining operations). Engineers from the Ministry of Transport are installing a liquid-nitrogen cooling system.

The damaged reactor seems to be cooling very slowly; on 6 May, the Ukrainian Prime Minister Aleksandr Lyashko said that the temperature had fallen to around 300°C, but ten days later,

Academician Velikhov cited the same figure. Also, the "sarcophagus" being built around the damaged reactor is not a simple casing, but, according to Silaev, is a "complex engineering structure", which will "fully control the internal heat and release any excess".

Not only the damaged reactor, No.4, will be entombed in this way. According to Prime Minister Lyashko, the unfinished reactor No.5, where shoddy building operations had caused concern before the accident, is also to be buried.

Away from the reactor site, but still within the 30-km evacuation zone, some measures have been implemented to safeguard agriculture. By 13 May, barrages had been built to stop effluent or rainwater reaching the river Pripyat. The main problem is the contaminated soil, which is being dealt with mechanically, by digging it out, and by planting with crops that "neutralize harmful elements in the soil".

To deal with long-term contamination further afield, an aerological monitoring station has been established, some 50 km from Chernobyl, which will make round-the-clock measurements of the radioactive contents of the air, and relay aggregate figures to the International Atomic Energy Agency in Vienna daily.

Little has emerged about radiation levels near the plant. By mid-May, according to transport construction minister Vladimir Brezhnev, radiation was still too high to work more than a four- or at most five-hour shift near the reactor, while the youth Party daily *Komsomolskaya Pravda* spoke of the emergency workers receiving a year's maximum permissible dose in two weeks. **Vera Rich**

Correction

In last week's News item about Dr Robert Gale's treatment of patients from the Chernobyl nuclear accident (*Nature* 321, 369; 1986), the number of patients who received bone marrow or fetal liver transplants was incorrectly given as 35; the figure should have been 19. The estimated average radiation dose of the 35 patients Dr Gale examined is 2 Gy or more; the estimated average dose of the 19 who received transplants was 5 Gy or more. □

SERC failing astronomers?

SIR—As a senior member of the research staff of the Royal Greenwich Observatory (RGO), I found your News article "Observatory move stirs passions" (*Nature* 321, 4; 1986) most interesting. At RGO I am somewhat of a "dove" about the move because I am concerned that a refusal to accept the decision of the Science and Engineering Research Council (SERC) in this matter, particularly on political grounds, could in the long term be bad for UK science. It may be better to accept a bad decision made by scientists now, in the hope of good decisions by scientists in the future, than to give ourselves over to overtly political decisions for ever.

Why does the decision seem so bad and arouse so much passion? Primarily because the stated reasons make no sense and are widely perceived to be a cover for a cost-cutting exercise. Placing RGO in a more stimulating academic environment is a worthy goal, but it would command more credibility if the actions of SERC over the past few years (an overall rundown at RGO, a further cut of our research in favour of a support role and failure to support efforts to strengthen the existing link with the University of Sussex) were consistent with this objective.

The expectation that the move can be self-financing also seems questionable. How can it be self-financing compared, for example, to the unexplored option of selling the castle and grounds at Herstmonceux while retaining the observatory, which is mostly in a modern office block in one corner of the property?

Thus most people believe that the move is an administrative device to cut costs in optical astronomy to free funds for new activities. They are reinforced in this belief by the rundown of RGO over recent years and the failure of SERC to place any of its New-Blood positions in university departments specializing in optical astronomy (only fifteen went to astronomy as a whole).

Thus, as an optical astronomer who believes in the importance and intellectual validity of my subject, I was opposed to the decision for exactly the same reasons as it seems to be attractive to SERC.

I do not believe that there is any wickedness involved in the decision or the under-appreciation of optical astronomy; simply muddle. Two more examples of SERC's confusion appear in your article. First, the publication of the results of the poll of staff in RGO about the move: the survey was co-sponsored by the RGO staff side who had the privilege of seeing them for the first time in your pages. Second, the gymnastics by Harry Atkinson as portrayed in your final paragraph suggest that political considerations (at least of a regional nature) are indeed part of

SERC's desiderata.

I am sorry to say that all this makes my stated support of SERC's decision pretty thin. What is needed to get SERC off the hook is an independent review as sought by the East Sussex County Council and preferably along scientific lines. I propose as chairman of this review the vice-chancellor of the University of Sussex, Sir Denys Wilkinson, who should be joined by a world-renowned foreign optical astronomer and a leading representative of our own (non-political) astronomical community. Such a group might have the moral authority to persuade everybody to accept its decision.

It is clear to me, however, that the organization most in need of serious investigation is the Swindon headquarters of SERC. It has shown itself much more eager to control the minutiae of activities in its laboratories, such as RGO, than to try to bring new resources into (in particular) a pure science like astronomy. From my perspective, they have singularly failed me, and other active scientists whether in universities or in their own laboratories, in this regard.

MICHAEL PENSTON

*Rose Lodge,
Magham Down,
Hailsham,
E. Sussex, BN27 1PR, UK*

SIR—As representatives of the staff of the Royal Greenwich Observatory (RGO), we read with interest your analysis (*Nature* 321, 4; 1986) of the current debate on the future location of the Observatory. Even though it is our lives and careers that will be affected by the outcome of this debate, we feel we are but pawns in the Science and Engineering Council (SERC)'s game. For example, SERC has refused to allow the report of the Kingman panel to be circulated among RGO staff, although it is obvious that members of the press and others have had copies. We also learnt of the results of the survey of RGO staff for the first time in your columns, and although this has been traced to an administrative bungle rather than a deliberate act, it demonstrates SERC's scant regard for the people who will be most involved. Staff have been asked to state their preferences, but in view of all that has gone before they wonder how much notice will be given to their opinions.

We have now received the results of the staff survey but interpret the numbers very differently from your informant. In the first place, the questions did not even mention the possibility of remaining at Herstmonceux. Almost all staff are strenuously opposed to *any* move and have said so consistently and vociferously from the outset. Had you been present

when the chairman of SERC, Professor E.W.J. Mitchell, met staff at Herstmonceux on 1 May, you too would have been in no doubt of their views. Second, the apparently high percentage who preferred Cambridge accounts for only 40 of the 119 "mobile" staff (out of the present total staff of 190 or so): replies from "mobile" staff who said they would resign rather than move anywhere and from "non-mobile" staff who face redundancy as a result of any move, were excluded. Staff who said they would follow the observatory wherever it went were added to the Cambridge-first-choice group. Fifty-five of the 119 did not reply at all to the questionnaire (many because they were fearful that the results would be misused). What price statistics?

Our strong desire to remain in Sussex is not blindly reactionary. If we could be convinced that there were clear and quantifiable benefits to be gained from moving to a different environment, we would welcome the proposal and consider the arguments on their merits: instead we are offered grounds which the council's officers themselves describe as a "matter of judgement" and "intangible" which to us as scientists is just not good enough. RGO staff wish to participate fully in the bright future for ground-based astronomy which the present facilities offer. A futile move would place this future in jeopardy and we urge our many supporters in the astronomical community to convey their views to SERC before it is too late.

JANET DUDLEY
(Chairman, RGO Staff
Trades Union Side)

*Royal Greenwich Observatory,
Herstmonceux,
Hailsham, Sussex BN27 1PR, UK*

Ageism

SIR—Jürgen Neffe (*Nature* 320, 295; 1986) writes: "Although now 74, von Weizsäcker's opinion is still influential in West Germany." The probably unintentional pejorative use of "Although now 74..." implies that the influence of an opinion should diminish with advanced age of the opiner.

This is an outdated, incorrect ageist myth. It is out of place in any publication intended to be a modern journal of science. I believe that an apology is due to Professor von Weizsäcker.

ARTHUR CHERKIN
*Geriatric Research, Education,
and Clinical Center,
VA Medical Center,
Sepulveda, California 91343, USA*

• The form of words correctly complained of was not that used by Jürgen Neffe but was introduced in the editing. — Editor, *Nature*.

Paranormal theories

SIR—While accepting Marks' general remarks about the standing of research into the paranormal (*Nature* 320, 119; 1986), I should like to comment upon some of his statements.

It is not true that there are "no theories to account for paranormal effects". In Bell's theorem and its refinements, in physics, we are confronted with a universe where local causality might not apply (for an entertaining discussion see Zukav, R. *The Dancing Wu Li Masters*; Fontana, London, 1984). This allows Jung's "synchronicity" and *psi* phenomena to be placed within a theoretical framework, although not suggesting a mechanism.

Theories such as those above are at the same time physical and metaphysical, as they question our basic concept of reality. It is not surprising that "believers" in the paranormal are resistant to change in their core constructs, and it seems trite to add that they have "significantly higher neuroticism scores". What does that prove? Is it Marks' view that these "millions of people throughout the world" are psychologically abnormal? One would assume that the whole review could be rewritten, with relevant changes, to cover "investigating God". Believers would, no doubt, react in a similar way, based on a similar "dearth of hard evidence".

In summary, I agree with Marks' conclusion that there have been no significant discoveries made by parascience, but I think that he need not have looked for psychological reasons for belief in the paranormal. As far as I can see (adjusting one of his phrases), scientific ideas will have little chance of affecting the magical thinking from which science itself evolved, because the fundamental mysteries remain.

M. COUCH

Department of Human Metabolism
and Clinical Biochemistry,
University of Sheffield Medical School,
Sheffield S10 2RX, UK

SIR—In his Commentary, Marks¹ misrepresents the area he purports to review. A large part of the paper refers to religious and neurotic motives as explanation for the belief in the paranormal. However, similar psychiatric explanations are used to account for the need to deny these phenomena^{2,3}. *Ad hominem* arguments lead nowhere.

As for Marks' more straightforward claims, they are indirectly based on a sample of works that is by no means representative. He relies on two reviews^{4,5} that do not address themselves to the question of repeatability in general, but rather to the specific question of whether parapsychological research involving altered states of consciousness constitutes a repeatable paradigm. Thus, these reviews

deal with a narrow group of experiments. For example, Akers⁴ excluded all studies of highly selected subjects, thus considerably decreasing the overall significance of the results. Moreover, Hyman's⁵ analysis is flawed, as Honorton has demonstrated in a response which followed Hyman's paper⁶, and his methods were shown by a statistician to commit the very error that he criticized in the parapsychological studies⁷.

Marks is quite right in pointing out that parapsychology has so far failed to produce a repeatable experiment. But it is misleading to use this as an argument against the credibility of parapsychological research. Marks fails to mention highly significant results of experiments that are characterized by extraordinary instrumental sophistication and methodological rigour, as exemplified by the recent work of R.G. Jahn of Princeton University⁸, and the Maimonides Hospital dream researches⁹⁻¹³. These works, although not yet replicated, pose a serious challenge to the sceptic.

There are four counterhypotheses by which parapsychological findings can be explained away: (1) coincidence; (2) artefact; (3) self-deception; and (4) deliberate fraud. Now, the growing sophistication and rigour of modern *psi* research¹⁴ is gradually ruling out the first three counterhypotheses. The fraud hypothesis (or, considering the present number of scientists involved in the field, the conspiracy hypothesis) remains as the only resort for the sceptic.

Psi or fraud? This is the real issue. Yet it is seldom presented unambiguously. Recently, Child¹⁵ has thoroughly analysed the ways in which the above parapsychological works have been treated in the sceptical literature. He has shown that they were either ignored or grossly misrepresented, and that the resort to the accusation of fraud was often made implicitly. It is deplorable that Marks' review, written after Child's grave charges have been issued, commits the same errors.

AVSHALOM C. ELITZUR

15 Margolin St,
Rehovot 76225,
Israel

1. Marks, D.F. *Nature* 320, 199 - 124 (1986).
2. Greyson, B.J. *J. nerv. ment. Dis.* 165, 184 - 200 (1977).
3. Eisenbud, J. *Parapsychology and the Unconscious* (North Atlantic Books, Berkeley, 1983).
4. Akers, C. in *Advances in Parapsychological Research* Vol. 4 (ed. Krippner, S.) 112 - 164 (McFarland, Jefferson, North Carolina, 1984).
5. Hyman, R. *J. Parapsychol.* 49, 3 - 49 (1985).
6. Honorton, C. *J. Parapsychol.* 49, 51 - 91 (1985).
7. Saunders, D.R. *J. Parapsychol.* 49, Appendix B (1985).
8. Jahn, R.G. *Proc. IEEE* 70/2, 136 - 170 (1982).
9. Krippner, S. & Ullman, M. *J. nerv. ment. Dis.* 151, 394 - 403 (1970).
10. —, Honorton, C. & Ullman, M. *J. Am. Soc. psychosom. ment. Med.* 20, 9 - 17 (1973).
11. Ullman, M. *Exp. Med. Surg.* 27, 19 - 38 (1969).
12. —, Krippner, S. & Feldstein, S. *Int. J. Neuropsychiat.* 2, 420 - 437 (1966).
13. — & Krippner, S. *Biol. Psychiat.* 1, 259 - 270 (1969).
14. Wolman, B.B. (ed.) *Handbook of Parapsychology* (Van Nostrand Reinhold, New York, 1977).
15. Child, I.L. *Am. Psychol.* 40, 1219 - 1230 (1985).

Pesticide dangers

SIR—J. Gordon Edwards (*Nature* 320, 391; 1986) has misunderstood Lord Ashby's review of *Pesticides and Nature Conservation: The British Experience 1950-1975* by John Sheail (*Nature* 318, 21; 1985); I cannot believe that he has read the book.

I agree that members of the "loony left" of the conservation movement deserve J. Gordon Edwards' strictures, and I have myself criticized them for the past 30 years. But John Sheail's book describes the way in which scientific work in pesticides in Britain generally resulted in collaboration between industry, government and responsible conservation bodies, to introduce sensible controls of potentially dangerous chemicals. As director of the Nature Conservancy's Monks Wood Experimental Station at the time, I know that my colleagues certainly did not "resort to such unscientific methods as deliberately distorting or omitting all the data that refuted their allegations". The work has, of course, been published in reputable scientific journals.

Lord Ashby in his review implies that Rachel Carson's *Silent Spring* alerted the authorities and the public to the dangers of pesticides. That danger was already fully recognized, at least among scientists. In fact, in Britain the most important controls of pesticides that had been shown to have harmful ecological effects were introduced, with the fullest cooperation of the chemical industry, some months before *Silent Spring* was published.

KENNETH MELLANBY

Monks Wood Experimental Station,
Huntingdon PE17 2LS, UK

Ling defended

SIR—The ill-natured and unnecessary sideswipe at Gilbert Ling (*Nature* 320, 318; 1986) by Peter Newmark needs to be answered. Ling, "a physiologist... who remains a voice in the wilderness", is the man whose pioneer work with Gerard on intracellular potentials initiated just about all the advances in neurophysiology of the past fifty years. Is it possible that Ling knows something about cell interiors that his critics are missing?

As for the sodium pump, Newmark could consult our papers in *Nature*¹ which by some miracle escaped the greater excommunication. To my knowledge, no other papers critical of the peculiar and unlikely hypothesis of active transport "powered" by metabolic pumps have ever been accepted and published by either *Nature* or *Science*. Such unanimity in science is unreal.

H. R. CATCHPOLE

110 West Oak Street,
Chicago, Illinois 60610, USA

1. Joseph *et al.* *Nature* 191, 1175 (1961); 203, 931 (1964); 206, 6 (1965); 209, 398 (1966).

Prehistoric ecology from pollen record

SIR—A “settlement gap” implied by the Weser estuary pollen records circa AD 500–700 does not pose “a fresh problem”, rather it supports an old conclusion. This is a protohistoric period significantly called the Migration Period (*Völkerwanderungszeit*) and virtually every settlement and cemetery in the western half of the Weser–Elbe “triangle” was abandoned in the first half of the fifth century, while those in the eastern half continued into the sixth century². Mass emigration brought many of these people, who called themselves Saxons, to eastern and southern Britain. The artefacts buried in their new settlement and cemeteries here link directly back to the Weser–Elbe homeland.

There is no need to invoke plagues or other sudden catastrophes, for a steadily rising sea level left the coastal marshland settlements on their *Wurten*, for example Feddersen Wierde³, without sufficient agricultural land and the inland settlements on the *Geest*, for example Flogeln⁴, appear to have reached a point of marginal returns from their over-cultivated soils. The Venerable Bede summarized the historical basis for all this in the eighth century AD in Book I Chapter 15 of his *Ecclesiastical History of the English Peoples* and both archaeology and the environmental sciences appear to confirm his account.

MARTIN G. WELCH

Department of History (Medieval Archaeology),
University College London,
Gower Street, London WC1E 6BT, UK

1. Moore, P.D. *Nature* **319**, 361–362 (1986).

2. Böhme, H.W. in *Führer zu vor- und frühgeschichtlichen Denkmälern* 29: *Das Elb-Weser-Dreieck*, pt.1, 205–225 (Philipp von Zabern, Mainz, 1976) esp. map on p. 223.

3. Schmid, P. in *Lowland Iron Age Communities in Europe* (eds Cunliffe, B. & Rowley, T.) 123–145 (Oxford, 1978).

4. Zimmerman, W.H. in *Lowland Iron Age Communities in Europe* (eds Cunliffe, B. & Rowley, T.) 147–163 (Oxford, 1978).

Nuclear risks

SIR—After reading your leading article (*Nature* **320**, 384; 1986), the persecuted employees of the nuclear power business must wonder what they have to do to satisfy journalists and broadcasters. As I understand it, their industrial accident record, both in respect of injuries to their own operators and to the public at large, is among the best in either the fuel industries or the chemical industries, to which their activities have perhaps the closest similarity. What can you mean therefore by your reference to the “poor safety record of Sellafield”?

As to the supposed poor public relations activities, I am not sure that you and the press generally give them much attention. What can your victims do in the face of a persistent campaign based upon the

supposed occurrence of dangerous accidents for which there appears to be no evidence, when that same lack of evidence is the basis for claims of secrecy and “cover-ups”.

Is it not clear that the employee of the nuclear power business is, while at work, far less liable to accident or death than you are at the wheel of your car? Indeed, are not members of the public more at risk from you at the wheel of your car than they are from the nuclear power business? Drivers kill about 5,000 people each year in the United Kingdom, whereas handling radioactive products for nuclear fuels appears to kill nobody (not even, I believe, the shellfish living comfortably by that waste water outlet from Sellafield into the Irish Sea).

A.E. ROUT

73 Cambridge Road,
Middlesbrough,
Cleveland, TS5 5NL, UK

• The article complained of was intended to be ironical, as should have been apparent from the last sentence — Editor, *Nature*.

Sexist ads

SIR—I would like to protest at a number of recent advertisements that have appeared in *Nature* depicting members of the male sex as colourless white-coated individuals. Surely you must realize that many of your readers are men and take exception to this unfair treatment, especially when, in the same issue, several women are illustrated wearing fashionable clothing, doing interesting things like swimming and looking as if they are having a good time?

I am afraid I shall have to cancel my subscription forthwith.

M. CLARK

75 Agarose Grove,
London NW3, UK

Velikovsky's evidence?

SIR—Your more perceptive readers may have been surprised to read in C. Leroy Ellenberger's letter (*Nature* **318**, 204; 1985) the statement, attributed to me, that “if a formerly very bright comet periodically altered the sky's brightness, then the dates of appearances and disappearances for Venus would be affected”. Venus is such a brilliant object that variations in the brightness of the sky would make little difference to its setting and rising dates. “The debris from the disintegration of comet Encke 4700 years ago” might, of course, act indirectly by filling the stratosphere with enough dust to prevent Venus being seen near the horizon line. This would produce longer invisibility periods; but only in one year, or perhaps two separated by an interval corresponding to the period of the comet. It would not explain

all the anomalies of the Venus Tablets.

With regard to the Velikovsky theory, archaeological evidence from Babylonian clay tablets has not verified the “Comet Venus” identification; but a large comet other than Venus, as deduced by Clube and Napier in their “Cosmic Serpent”, is a possibility.

JOHN D. WEIR

72 Baronscourt Terrace,
Edinburgh EH8 7EP, UK

Plasma — the fourth state of matter?

SIR—J.N. Goldschvartz (*Nature* **320**, 302; 1986) basically asks “what is the fourth state of matter?”. There is no debate about the first three phases of matter¹ — solid, liquid and gas. When a solid is heated, a liquid is formed, and when a liquid is heated it is transformed into a gas; a commonplace example is the progression from ice to water to steam.

When a gas is heated, that is, when additional energy is provided to the atoms or molecules, some of the electrically neutral particles can be split into electrons and positive ions. Such an electrically charged gas, a partially ionized gas, which overall is electrically neutral, is termed a plasma if it exhibits “collective” (jelly-like) behaviour² due to the long-range (on an atomic scale) electromagnetic forces acting between the charged particles.

Clemmow and Dougherty³ say on page 1 that “the state in which there is an appreciable degree of ionization has indeed been called the fourth state of matter”. Surely plasma should, unequivocally, be termed the fourth state of matter.

MICHAEL J. RYCROFT

British Antarctic Survey (NERC),
Madingley Road,
Cambridge CB3 0ET, UK

1. Walton, A.J. *Three Phases of Matter* (McGraw-Hill, Maidenhead, UK, 1976).

2. Chen, F.F. *Introduction to Plasma Physics* (Plenum, New York, 1974).

3. Clemmow, P.C. & Dougherty, J.P. *Electrodynamics of Particles and Plasmas* (Addison-Wesley, London, 1969).

South Africa

SIR—In suggesting that South African scientists be required to sign a statement of political beliefs before their articles are considered by international journals or they themselves be allowed to attend overseas conferences (*Nature* **320**, 486; 1986), Masters, Caithness and Rayner respect neither science nor scientists. The quality of a work of science exists entirely within the work. Other requirements for publication or conference-attending are inherently corrupt and corrupting. And a man's political beliefs are nobody else's business unless he is a politician.

C.W. MCCUTCHEN

5213 Acacia Avenue,
Bethesda, Maryland 20814, USA

A child's guide to radiobiology

The view that radiobiology (made fashionable by the accident at Chernobyl) is unknown territory is belied by the vast literature on the subject. But uncertainties persist.

FEW subjects can have been given as much attention in the past thirty years as that of the biological consequences of radiation. The explanation is simply that governments have felt it necessary to make independent assessments of the consequences for their populations of the bulk production of large amounts of artificial radioactivity, which became a live issue in the late 1950s with the testing of nuclear weapons in the atmosphere. The result is that there is no shortage of information. Public reaction outside the Soviet Union to the consequences of the Chernobyl accident has nevertheless provided further proof that to publish is not necessarily to spread understanding.

That radiation may be damaging has been plain for more than half a century, most vividly by the evidence of the damage accidentally done to occupational groups such as the sad cohort of workers in the US watch-making industry employed to paint the luminous dials of watches and who kept their paint-brushes moist by licking them. In the 1920s, the liability to injury of people working with X rays used for medical diagnosis and therapy was recognized by the fixing of a supposed safety limit for exposure, defined as ten per cent of the dose (in roentgens) required to cause a disorder of the blood-forming process in adults.

Now, as the limits have been steadily reduced for the allowable exposure to radiation for occupational and other groups, there is also a reductionist framework to explain the rationale of the dose-fixing regulations. But that does not mean that the uncertainties have been banished.

The agents of the biological damage are, microscopically, a mixed bag, whose effects are best described in language more familiar in high-energy physics. Charged particles such as alpha-particles are stopped in matter of all kinds, living or dead, by the ionization they cause, although there may be occasional nuclear collisions as well. X rays and more energetic gamma rays are also chiefly sources of ionization. Electrically neutral particles, neutrons for example, interact to a comparatively negligible degree with the electrons in the outer shells of the atoms through which they pass, but are capable of colliding with nuclei, which is the simple reason why neutrons are relatively more damaging, by the yardstick of the amount of energy deposited in

irradiated material, than most other kinds of radiation.

Inanimate matter is not permanently damaged by radiation except to the extent that ionization of atoms and molecules may catalyse chemical reactions (which is why radiation may cause organic materials to polymerize) or that atoms may be displaced from their required positions by nuclear collisions (which is why irradiated graphite requires annealing).

Living cells are obviously more vulnerable. The mere deposition of external energy in the form of ion-pairs is an assault that may affect essential processes, killing damaged cells or, alternatively, like nuclear collisions at the wrong places in a vital molecule, causing mutations that, in somatic cells, may lead to cancer and, in germline cells, to genetic defects.

By what yardsticks should the potential of radiation for causing damage be assessed? In an ideal world, there would be two quite different bodies of information: first, a set of what physicists would call cross-sections to determine the chance that the passage of a particle (or photon) would set in train each possible biochemical process; and, second, a body of information about the biological consequences. Instead, there are two largely empirical sets of rules of thumb.

The equivalent of the set of cross-sections is the assumption that the damage done to a tissue by irradiation is proportional to the amount of energy deposited within it, whence the unit called the rad. The different components in a mixed source of radiation are weighted according to their "quality factors" to allow for the biological damage they do, by which process rads are translated into rems (for which the unit is the sievert).

Present practice will probably in due course seem crude. Because cross-sections are functions of energy, one would expect the quality factors of the same particles to be functions of energy as well. This point has emerged from analysis during the past few years of the incidence of genetic defects among the children of the survivors of the two bombs at Hiroshima and Nagasaki. Ultimately, no doubt, there will be different quality factors for the causation of genetic defect, cancer and more acute effects, and new units in which to measure them.

The other side of this coin, the numerical relationship of biological damage to its causes, measured as radiation doses, is

inevitably more crude. Part of the trouble is simply biological: a genetic defect may show up as a fetus that dies *in utero*, and the biological consequences may be negligible in a population living within its reproductive potential. That is why the working yardstick since the Second World War has been the assumption that, whatever the quantitative relationships may be, the natural dose of radiation to which we are exposed is, by definition, one with which a species must expect to be able to live.

The assumption is natural but its form has changed. Thirty years ago, cosmic rays were supposed to be the chief source of natural radiation; most of the components of cosmic rays, being fast, penetrate the whole body and deliver a reliably calculable dose of radiation, 0.30 millisieverts a year. The effects of naturally occurring radioactive species such as those of potassium-40, have always been recognized as important, but radon-222 now accounts for as great a general radiation dose as the other two put together. On the average, individual exposure to radiation for people living near sea-level is 2 millisieverts a year.

To use natural exposure as a measure of the risks to which people are artificially exposed, it would clearly be necessary that there would be quantities (not necessarily constants of proportionality) relating damage to dose. But it seems unlikely that simple numbers will ever be found. Different kinds of cancer may be induced by radiation with differing efficacy; some genetic defects may be more likely than others.

Complications such as these already have a bearing on some practical problems, especially where internally ingested radioactivity is involved. Although claims that the rate of genetic malformation among the children of people living near nuclear test sites in the United States, put forward by Dr David Sternglass of the University of Pittsburgh in the 1960s, have been shown to be statistically invalid, there is always a possibility that certain radionuclides may have biological consequences that are systematically underestimated by the measure in rems of the dose they deliver, perhaps because they concentrate in particular places. The difficulty here is that each investigation is a major undertaking, and that there are simply not enough people to go around.

John Maddox

Biomechanics

The flight of the dipteran fly

from H. C. Bennet-Clark

THE wing-beat frequency of flies in the order Diptera varies from 120 Hz in the larger species to more than 500 Hz in the smallest midges. These frequencies are far faster than can be maintained by conventional muscle, where there is one impulse per contraction. The flight muscles of dipteran insects are not excited synchronously in this way (Pringle, J.W.S. *J. Physiol., Lond.* **108**, 226; 1949) but vibrate at a far higher frequency. Attempts to model the processes in the dipteran thorax that cause flight have been based on a 'click' mechanism which can be elicited in anaesthetized flies. But recent experiments indicate that the click mechanism is an artefact and that the flight of flies *in vivo* involves a quite different mechanism (Miyan, J.A. & Ewing, A.W. *Phil. Trans. R. Soc. B* **311**, 271; 1985).

Early experiments by Pringle and others demonstrated that, when insect flight muscle is activated and subjected to rapid elongation, the subsequent response is a contraction after a brief delay and over a short distance. Thus, these muscles are stretch-activated and must be inserted in a skeletal system of considerable stiffness, with kinematics that provide an appropriate stretch to activate the antagonistic wing levator and depressor muscles at appropriate phases in the wing-beat cycle of the insect.

When flies are anaesthetized with CCl_4 they 'go under' with the wings either abnormally elevated or depressed. In this condition, with the wings in the raised position, the thorax can be squeezed longitudinally between forceps and the wings will then start to depress until, at about mid-position, they suddenly click into the fully depressed position. If the thorax is squeezed dorso-ventrally, the opposite slow movement occurs, followed by the click. This model mechanism (Boettiger, E.G. & Furchspan, E. *Biol. Bull. mar. biol. Lab., Woods Hole* **102**, 200; 1952) has two attractive features: it provides, at approximate phases in the wing stroke, stretch-activation for the major flight muscles; and, because of the geometry of the wing base, it automatically alters the angle of attack of the wing between the downstroke and the upstroke.

One snag with this model is that the

wing should behave like a toggle switch (*a* in the figure). In such a switch, force on either upstroke or downstroke opposes movement until approximately mid-stroke and then reverses to act in the same direction as movement, to complete it with a snap. In practice, this is only seen in the CCl_4 -anaesthetized flies: in normal flight, the wings of Diptera only decelerate near the top and bottom of the wing beat instead of in the mid-downstroke and again in the mid-upstroke. This simple click model has the deficiency that it does not take account of the force caused by the flight muscles, which tends to be maximal in the part of the stroke where maximal force has to be produced to overcome that resulting from the click mechanism.

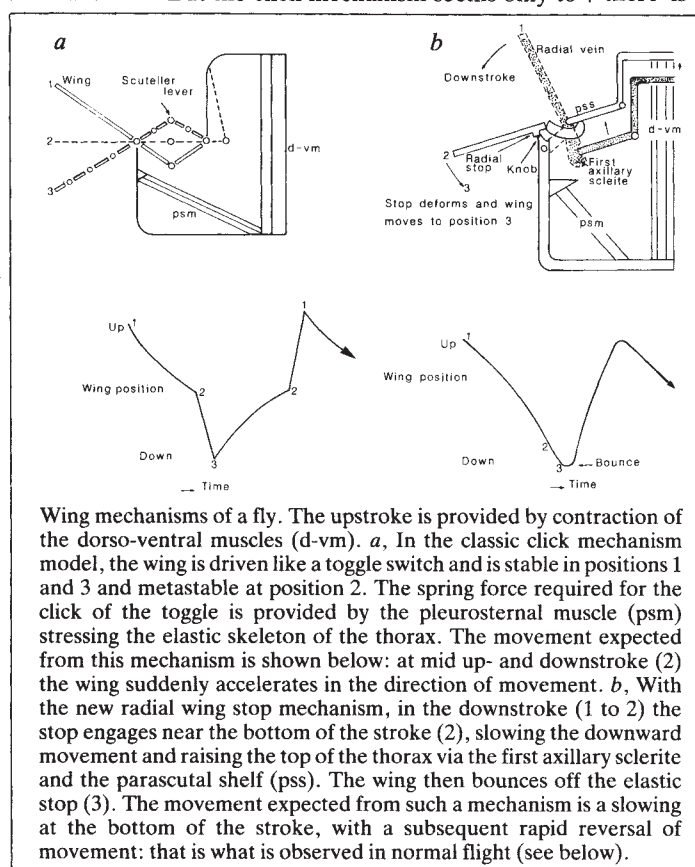
But the click mechanism seems only to

normal action of the dipteran thorax? In their new work, Miyan and Ewing studied in detail the thoraces of a dozen dipteran species, many of which were literally frozen in flight by being tipped into liquid nitrogen. They describe a stop under the base of the principal vein, the radius, which can engage on a knob at the crest of the side of the thorax (*b* in the figure). In the downstroke, when the wing stop engages, the inertia of the wing causes the outer part of the wing to bend downwards (seen in high-speed films of flight in many species) and, in addition, through the action of the first axillary sclerite and parascutal shelf in the wing base, causes a sharp lifting of the dorsal part of the thorax (*b* in the figure) which, in turn, stretches the dorso-ventral muscles to activate them for the ensuing upstroke. The wing is accelerated upwards only at the very start of the upstroke, probably because of the elastic recoil off the radial wing stop, and thereafter swings with nearly constant angular velocity, as would be expected if there is a steady torque caused by the

flight muscles (but which is against the predictions of the click mechanism) until the wing decelerates at the top of upstroke and then starts the downstroke (*b* in the figure). Vortex shedding at the bottom of the wing stroke will be enhanced by the rapid change of wing direction and angle of attack that the radial wing stop allows, which is compatible with the analysis of the unsteady-state aerodynamics in the flight of small insects made by T. Weis-Fogh (*J. exp. Biol.* **59**, 169; 1973). It is not clear whether there is a special mechanism at the top of the upstroke to snap-stretch and activate the muscles involved in the downstroke.

The older click mechanism model also allows the amplitude and/or frequency of the wing beat to be increased by contraction of the pleurosternal muscle (psm in the figure) increasing the spring force produced between the side of the thorax (at the outer wing hinge) and the inner, complex, wing hinge attached to the dorsal part of the thorax. The prediction from the click mechanism model is that this would act symmetrically on both the upstroke and the downstroke, but what is in fact observed is an asymmetrical effect, mainly affecting the downstroke, which can now be explained partly by the larger size of the dorsal-longitudinal wing depressor muscles compared with that of the dorso-ventral wing depressor muscles.

Miyan and Ewing also address the prob-



appear under CCl_4 anaesthesia, according to the recent work of Miyan and Ewing (*J. exp. Biol.* **116**, 313; 1985). In this special circumstance, the muscle that tenses the side of the thoracic box (psm in the figure) goes into such strong tetanus that the normal action of the sclerites of the wing base cannot occur — the click mechanism thus appears to be an abnormal artefact of the anaesthetic.

What then does happen during the

lems of control of the plane and form of the wing beat by the various skeletal and muscular elements. Their description of the action of the sclerites in the wing base allows both the continued beating of one wing when the other is folded back, as seen in the rapid turning of bluebottles, and also fine control of wing twisting, neither of which is easy to explain using the old model. These manoeuvres are effected by contraction of various of the 17 distinct, normally activated, non-fibrillar

muscles in each side of the thorax. The system is amazingly complex: the wing base is the region at which the stresses are highest but it allows both control of the wing beat and the wing to be folded back, both when the insect is at rest and when the other wing is beating. □

H.C. Bennet-Clark is Lecturer in Invertebrate Zoology in the Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

Nuclear magnetic resonance

Protein structure determination

from Wolfgang Kabsch and Paul Rösche

ONE of the most fruitful areas of biophysics is the elucidation of the three-dimensional structures of biologically active molecules such as proteins and nucleic acids in atomic detail. These investigations have brought a wealth of new information on the structural design principles of these molecules and their functions and interactions. Two papers published in the 20 May issue of the *Journal of Molecular Biology*^{1,2} report the three-dimensional structure of the 75-amino-acid-long α -amylase inhibitor by two different methods: Pflugrath *et al.*¹ used the classical method of X-ray crystallography, whereas Kline, Braun and Wüthrich² derived the three-dimensional structure of the protein entirely from proton nuclear magnetic resonance (¹H-NMR) measurements in solution. The overall features of the X-ray structure and the NMR structure seem to be very similar, confirming that NMR gives an accurate measure of protein structure.

Until recently, the only method for determining macromolecular structure at atomic resolution was X-ray crystallography. For this technique to be applicable, single crystals of the molecules have to be grown to linear dimensions of at least 0.2 mm. In addition, to resolve the phase ambiguity of the scattered X-rays, isomorphous heavy-atom derivatives of these crystals are required. For proteins up to a relative molecular mass (M_r) of about 50,000, structure determination is a straightforward application of standard techniques as long as the amino-acid sequence is known. Unfortunately, it turns out that not all biologically interesting molecules can be obtained in crystalline form. Although new methods are constantly being developed, crystallization and selection of suitable heavy-atom derivatives remain an art in themselves.

The paper by Kline *et al.* is a remarkable step forward in the biochemical application of NMR. Since the 1950s, ¹H-NMR has delivered structural information of small molecules predominantly by its

main parameter, the chemical-shift value. Even in the mid-1970s it was generally thought that structural information on biological macromolecules could be obtained, if at all, only by retrieving the information hidden in the shift values of the proton resonances, as for technical reasons no other parameter seemed directly available from the NMR spectra. An obstacle which had to be surmounted anew for each protein molecule was the assignment of resonances to the chemical groups of the individual amino-acid residues in the protein. This task is formidable, considering that even a small protein of $M_r \sim 10,000$ (corresponding to about 90 amino acids) already gives approximately 500 different resonance lines. In addition, most of these lines are split into multiplet structures. Thus it seemed that what was needed for ¹H-NMR to become generally useful in structure determinations was first, a method for the sequence-specific assignment of resonances; and second, a method for the retrieval of structural information from chemical-shift information. Ten years ago the possibility of achieving at least the first goal, let alone the second, seemed remote. The gradual improvement in available magnetic field strength brought about no substantial change — nor did anybody seriously expect it to do so.

Thus, an entirely new approach was needed. It originated in 1971, when Jeener³ supplied a basic idea seemingly remote from practical applications in the general NMR laboratory. In Fourier NMR (today the standard way of obtaining NMR spectra) the decay of nonequilibrium magnetization after application of a high-frequency pulse is observed as a function of time. This function yields, after Fourier transformation, the desired spectrum, that is, the absorbance of the sample as a function of frequency. Jeener's idea was to substitute the single high-frequency pulse with two pulses of variable pulse separation, thus introducing a second time parameter. Two-dimensional

Fourier transformation then yields a matrix with two frequency axes. Resonances on the diagonal correspond to the normal one-dimensional spectrum and off-diagonal resonances originate from the mutual interactions of protons; lines parallel to the two frequency axes through an off-diagonal resonance connect the two on-diagonal resonances originating from the two protons whose interactions gave rise to this off-diagonal peak. It was the achievement of Ernst and co-workers⁴ to recognize the great practical importance of this technique. His group and that of Freeman, among others, developed a wealth of new experiments based on this basic idea (see, for example, ref. 5).

What change did this bring about for biochemical applications? The advantage of two-dimensional over one-dimensional spectroscopy is twofold: first, the basic two-pulse two-dimensional experiment (later termed COSY, from correlated spectroscopy) allows resolution of connectivities of resonances belonging to groups covalently connected with a spacing of only a few bonds. The off-diagonal peaks are generated by 'through-bond' interaction of two protons — in general, a distance of not more than three bonds between the protons is required. This yields, at least in principle, the assignment of all resonances to types of amino-acid residues with one single experiment, because each amino acid is characterized by a specific fingerprint of proton connectivities. In practice, some additional information from other types of two-dimensional experiments may be required. Second, a three-pulse variant of the basic COSY experiment (termed NOESY, from nuclear Overhauser effect spectroscopy) yields information on spatial distances of groups closer than about 0.5 nm via the dipolar 'through-space' interaction of those groups. Thus, the resonances in a two-dimensional spectrum originating from directly neighbouring NH and C _{α} H groups and C _{α} H-CO-NH groups in the peptide bond are connected via off-diagonal resonances generated by the through-space interaction of those protons. The backbone resonances may then be traced according to the chain of amino acids in the protein by following the chain of -NH-C _{α} H-CO-NH- backbone proton resonances. The corresponding side groups are then identified in the COSY spectrum or via the NH-C _{α} H dipolar interaction in the NOESY spectrum. In addition, the distances between protons belonging to groups closely spaced in the three-dimensional structure — but not necessarily in the sequence — can be estimated. This yields information in the three-dimensional folding of the protein. Unfortunately, the spread of the correlation times for chemical groups in proteins prevents accurate determination of these distances via the nuclear Overhauser

effect. Thus, in general, only upper bounds on distances may be obtained by this method.

Thus, a set of two-dimensional spectra of a small protein generates a set of distance constraints for neighbouring groups. If a nuclear Overhauser effect is detected, the interproton distance is judged to be closer than, say, 0.4 nm. If no such effect is detected, the interproton distance can have any value. The potential of this method for structure determination of proteins was first realized by Wüthrich and co-workers⁸, but the methods for structure calculations from the knowledge of a set of proton-proton distance constraints are by no means trivial, and much work went into setting-up computer programs to this end. Evaluation of NMR data with the aid of programs such as those introduced recently by Havel and Wüthrich⁶ and Braun and Go⁷ may finally result in the elucidation of a few possible tertiary structures.

Kline, Braun and Wüthrich² have now gone one step further in the interpretation of their experimental results by grouping their measured nuclear Overhauser effects into three categories: large, medium and small proton-proton separations. They then assigned numerical values to these categories by comparison with the magnitude of the effects observed for protons with covalently fixed distances. As their result shows, the approach works for the α -amylase inhibitor structure, and will thus probably work for most other protein structures.

In the past five years the secondary structures of several polypeptide strands have been determined by these ¹H-NMR methods^{9,10}. As an example of a larger unit, secondary structure elements have been determined for the DNA-binding *lac*-repressor headpiece and a low-resolution model of its tertiary structure has been suggested¹¹. Of course it helped that the tertiary structures of other DNA-binding proteins, such as the CAP of *Escherichia coli*, were known from the start¹². The *lac*-repressor headpiece was until now the best example of a protein structure determined at low resolution by NMR methods, although the structural

model has so far not been verified directly by X-ray studies. For the *lac*-repressor headpiece, extensive use has been made of molecular dynamic calculations as a further method of secondary structure refinement¹³, a method that could also strongly influence protein structure determination by NMR methods in the future.

The paper by Kline *et al.*² is a step forward in the application of NMR to three-dimensional protein structure determination, but too much enthusiasm would be premature. The conditions under which NMR studies can reveal secondary structures or complete tertiary structures of proteins are rather restrictive: the protein must be sufficiently small ($M_r < 10,000$) to allow assignment of most resonances; soluble to high concentrations (several millimolar) to yield sufficiently good signal-to-

noise ratios; available in comparatively large amounts (tens of milligrams); and stable in solution for several days at elevated temperature and preferably low pH (less than 6) to allow observation of exchangeable NH backbone protons. At present, the determination of protein structures by X-ray analysis is still much more straightforward as long as good crystals are available. Nevertheless, we expect NMR to become a powerful tool in the foreseeable future for determining the structure of small- to medium-sized peptides and hormones, which are in any case very sensitive to heavy-atom substitution and are often not crystallizable at all. □

Wolfgang Kabsch and Paul Rösche are in the Department of Biophysics, Max-Planck Institute for Medical Research, 6900 Heidelberg 1, FRG.

Rock mechanics

Luminous phenomena and their relationship to rock fracture

from John S. Derr

INVESTIGATIONS of earthquake lights are moving from the gathering of anecdotal sightings into the laboratory, and in the process gaining a measure of respectability. Most recently, Brady and Rowell report on page 488 of this issue¹ that breaking rocks in the dark produces lights whose spectra are those of the ambient atmosphere. The experimental design is intended to help discriminate among several possible mechanisms for the lights, including frictional heating to incandescence; sparks caused by the piezoelectric effect; or charge separation, plasmas and charged particle bombardment. The authors show, for example, that rock fracturing under water produces spectra of both atomic and molecular hydrogen, and for this and other reasons conclude that earthquake lights are caused by exoelectron excitation of the ambient medium rather than by any of the other suggestions.

One of the early giants of seismology, Perry Byerly, enjoyed telling students that the subject of earthquake lights was the darkest area of seismology. The reason for its neglect was probably that most observations have been highly anecdotal in nature, made by untrained observers and contain little or no 'hard' data. To make matters worse, sightings of clear examples of earthquake lights and ball lighting are generally confused with even more controversial subjects like UFOs. Typical reports of earthquake lights can be found in the 'grey' literature and the popular press — no wonder scientists have been reluctant to touch the subject!

The phenomena have been reported for

centuries. George Frederick Handel created an imaginative rewrite of two biblical passages for his oratorio "Israel in Egypt"

He gave them hailstones
for rain: fire mingled
with the hail ran along
upon the ground.

This is probably a description of ball lighting, but is typical of common misperceptions which read religious or mystical interpretations into the sightings. Other examples include a recent sighting of luminous phenomena along fault lines in the United Kingdom which was interpreted as "luminous beings"². Tibetan monks have considered regularly occurring mountain-top lights to be a manifestations of the Bodhisattva³. These accounts are, of course, subject to many interpretations, but I find them at least consistent with the hypothesis that these people were astonished at the sight of luminous phenomena and gave the unknown a religious interpretation.

There is a very graphic Japanese haiku of unknown origin and time:

The earth speaks softly
To the mountain
Which trembles
And lights the sky

This suggests a general awareness of the phenomenon which may have led two Japanese seismologists, Terada and Musya, to initiate the modern study of earthquake lights in the early 1930s. The immediate stimulus was certainly sufficient: the Idu peninsula earthquake of 26 November 1930 was accompanied by lights brighter than moonlight⁴. Similar

1. Pflugrath, J. W., Wiegand, E., Huber, R. & Vertesy, L. *J. molec. Biol.* **189**, 383 (1986).
2. Kline, A. D., Braun, W. & Wüthrich, K. *J. molec. Biol.* **189**, 377 (1986).
3. Jeener, J. *Ampere International Summer School II* (Basko Polje, Yugoslavia, 1971).
4. Aue, W. P., Bartholdi, E. & Ernst, R. R. *J. chem. Phys.* **64**, 2229 (1976).
5. Bodenhausen, G. *Progr. NMR* **14**, 137 (1981).
6. Havel, T. & Wüthrich, K. *Bull. Math. Biol.* **46**, 673 (1985).
7. Braun, W. & Go, N. *J. molec. Biol.* **186**, 611 (1985).
8. Wüthrich, K., Wider, G., Wagner, G. & Braun, W. *J. molec. Biol.* **155**, 311 (1982).
9. Williamson, M. P., Marion, D. & Wüthrich, K. *J. molec. Biol.* **173**, 341 (1984).
10. Wemmer, D. & Kallenbach, N. R. *Biochemistry* **22**, 1901 (1983).
11. Zuiderweg, R. J. P., Kaptein, R. & Wüthrich, K. *Proc. natn. Acad. Sci. U.S.A.* **80**, 5837 (1983).
12. McCray, D. B. & Steitz, T. A. *Nature* **290**, 744 (1981).
13. Kaptein, R., Zuiderweg, R. J. P., Scheek, R. M., Boelens, R. & van Gunsteren, W. F. *J. molec. Biol.* **182**, 179 (1985).

descriptions came from the great Tangshan earthquake in China on 27 July 1976: people were woken up, thinking that someone had turned the lights on¹. Other typical descriptions of earthquake lights include a glow at sea like a ship on fire; small, sequential flashes from different, random places on a hillside; fireballs or meteors; and auroral streamers diverging from a point on the horizon¹.

Probably closely related to the lights are sightings of luminous phenomena which have many of the same characteristics and occur in the same areas as earthquakes, but not at the same time. The best-documented examples come from the Yakima Indian Reservation, Washington², where photographs and extensive written records permit statistical analysis. A typical description compares the luminous phenomena with a bright white light the size of a baseball floating among the tree tops. The results in that study support the hypothesis that the lights are associated with tectonic strain in active seismic areas and tend to accompany increases in earthquakes. Thus, earthquake lights and luminous phenomena may be related phenomena with slightly different time relationships to the associated earthquakes, or else luminous phenomena may, in fact, be earthquake lights for very small, high stress-drop earthquakes that have not been detected because of a lack of instrumentation.

The work of Brady and Rowell is a significant step because it discriminates between several proposed mechanisms and demonstrates that luminosities can be produced by any instrumentally recorded earthquake, even of the lowest magnitude. It strongly suggests that luminous phenomena are indeed earthquake lights produced by microearthquakes. Before this work, it was supposed that rock fracture produced plasmas which would generate easily detected microwave emissions. However, only a low frequency ($\sim 10^3$ Hz) was detected in these experiments³, a result which agrees with general observations during earthquakes. (Microwave emissions across the fault plane are consistent with this theory, but very little should be observable at the surface.) Also, it was not possible to explain earthquake lights at sea, except by invoking the help of legions of excited, phosphorescent plankton. By actually generating lights underwater, Brady and Rowell have advanced a reasonable mechanism for these sightings. This work is, therefore, the first fully successful laboratory demonstration of a possible mechanism for this phenomenon.

If microwave interference could be detected from an earthquake, a different mechanism involving spark discharge would be required. King and Freund⁴ show that nominally anhydrous minerals which dissolve small amounts of OH

during crystallization eventually develop peroxy anions (O_2^{2-}) and hydrogen molecules (H_2). With heat of decompression, unbound O^- charge carriers can be generated. Extremely high local electric fields would result, with dielectric breakdown of air and strong microwave emissions if the density of O^- ions exceeds about 20 parts per million. The dielectric breakdown and exoelectron mechanisms can be differentiated by the presence or absence of significant microwave radio noise, although both mechanisms can produce lights and should generate radiation in the 900- to 5,000-Hz range⁵.

Laboratory investigations of earthquake lights are just beginning, and the work of Brady and Rowell suggests many areas for investigation. Both exoelectron and dielectric breakdown mechanisms can explain the lights only if cracks extend to the surface in the area of the actual sightings. If a fault break does not reach ground level, one would require either a continuous series of cracks to provide a path for gas flow; or sufficient secondary fracturing at the surface to generate lights away from the primary fault. There is also a problem of timescale: to date, no experiment has produced any luminosity lasting even as long as one second. All laboratory tests should include broadband electromagnetic spectrum analysers to detect frequencies from ~ 10 Hz to X ray (10^{17} Hz).

Similarly, field investigations are in their infancy — there are great gaps in our knowledge. No one has tried to obtain pictures of earthquake lights triggered by nearly simultaneous signals from both a seismometer and photometer, or pictures and other measurements of lights in mine tunnels crossing faults. We do not know how exoelectron bombardment could produce a stationary light of relatively constant luminosity for as long as one hour, nor, as observed in the field, can we explain what would cause an image on infrared film that was not visible to the eye

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Earthquake lights from Matsushiro, Japan, near Mount Kimyo on 26 September 1966 at 0325 JST. The luminosity lasted for 96 s. Direction, due north. Photograph by T. Kuribayashi.

when the picture was taken⁶. Further study may reveal luminous phenomena to be a useful geophysical prospecting tool if they are related to mineralized bodies, or for earthquake prediction if we can understand the relationship between the area of sightings and subsequent earthquakes. Very preliminary correlations suggest that stress propagates across a geological terrane, causing luminous phenomena in the strong areas as it moves and earthquakes at the weaker boundaries⁷. In general, however, we remain ignorant of the necessary and sufficient conditions for generation of luminous phenomena and earthquake lights.

Brady and Rowell also suggest that their work has implications for biogenesis. It would be interesting to perform a Miller-Urey experiment using rock fracture as the electrical source to see if amino acids can be generated from primeval components under water or otherwise independent of atmospheric electricity.

Hence, a new, challenging area of geophysics is just opening. Previous studies have been criticized as statistical analyses without contributing towards finding a physical explanation. Brady and Rowell's work is a significant step to finding one such mechanism for the production of geophysical luminosities. But investigations in other areas are still required because we may be looking at several phenomena which sometimes share a common appearance and name. □

1. Brady, B. T. & Rowell, G. A. *Nature* **321**, 488 (1986).
2. Devereaux, P. *Earth Lights* (Turnstone, 1982).
3. Govinda, A. *The Way of the White Clouds* (Shambhala, 1970).
4. Derr, J. S. *Bull. seism. Soc. Am.* **63**, 2177-2187, 1973.
5. Lomnitz, C. & Lomnitz, L. (reported by Malcolm, A. H. *New York Times* 2 June 1977).
6. Derr, J. S. & Persinger, M. A. *Experientia* (in the press).
7. Cress, G. O., Brady, B. T. & Rowell, G. A. *Geophys. Res. Lett.* (in the press).
8. King, B. V. & Freund, F. *Phys. Rev.* **B29**, 5814 (1984).
9. Wiedenmann, C. L. *Vestiga Newslett.* **2**, 1 (1977).
10. Persinger, M. A. & Derr, J. S. *Perceptual and Motor Skills* **61**, 807 (1985).

John S. Derr is at the Geological Survey, US Department of the Interior, Box 25046, Denver Federal Center, Denver, Colorado 80225, USA.

Pattern formation

Segmentation genes and the distributions of transcripts

from Douglas Coulter and Eric Wieschaus

RESEARCH on the fruitfly *Drosophila* during the past five years shows that much of the global character of the insect's segmental pattern, as well as its finer details, depends on gene activity in the embryo. One of the attractions of segmentation as a system for studying pattern formation is that most of these zygotically active genes have been identified and their phenotypes characterized in mutant embryos¹. In the past year several of the genes have been cloned, so it is now possible to compare the actual patterns of gene expression at the blastoderm stage with the altered segmentation observed in mutant embryos. Recent results, reported on page 493 of this issue², suggest that mutant phenotypes and transcript distributions do not always match.

The common feature of all the mutations isolated so far is that they cause reductions in the overall pattern. In what is perhaps the most relevant class (the 'pair-rule' mutations) the pattern deletions are about one segment in size and are repeated at double-segment intervals along the length of the animal (see figure). Although each of the nine pair-rule loci is associated with its own precise deletion pattern, the domains affected by different genes overlap. A given element of the final pattern thus requires a defined subset of pair-rule gene activities for its formation. The deletion phenotypes observed in mutant embryos apparently arise because in the absence of any one of these activities, the cells that make those pattern elements die or assume alternate fates.

If wild-type genes for each locus are required only to form specific subsets of the final pattern, one might expect that at the blastoderm stage the respective gene activities would be found only in the precursor cells for those pattern elements. After the first pair-rule gene, *fushi tarazu* (*ftz*), was cloned^{3,4}, the distribution of transcripts was determined by using *in situ* hybridization to sectioned blastoderm embryos⁵. The transcripts were localized in seven stripes which seemed to correspond to the seven deletions observed at double-segment intervals in mutant embryos (see figure). This very exciting result suggested that the patterning mechanism in *Drosophila* might be decipherable strictly in terms of localized gene activity at the blastoderm stage.

Because each of the nine pair-rule loci produces its own pattern of deletions, it is difficult to argue that any mutant domain defines a unit more fundamental than any

other domain. This is contrary to what one would expect if the repeating pattern was established immediately as a series of units or 'compartments', because, if this were true, realms of gene expression would be expected to coincide with compartment boundaries. At first glance, it seems that the range of phenotypes is more compatible with some combinatorial or hierarchical model assigning fates to individual cells⁶ rather than to cell groups or polyclones⁸. Compartments could then be established later in response to the identities assumed by individual cells. Inherent to any of these models, however, is the assumption that one can actually extrapolate from the overlapping phenotypes in mutant embryos to actual gene requirements at the blastoderm stage.

This could only be tested when a second pair-rule gene, *hairy*, was cloned. Transcripts from *hairy* were shown to be localized in stripes with a width and periodicity similar to that of *ftz*⁹, but the phasing of the stripes was different for the two loci. When embryos were simultaneously hybridized with probes from both genes, four regions could be identified in each double-segment interval: cells which contained either *hairy* or *ftz*; cells which contained neither transcript; and cells which contained both. This overlapping distribution of transcripts appears to be analogous to the overlapping pattern deletions observed in some mutants at each locus⁷, suggesting that domains of gene expression at blastoderm correspond to domains of gene requirement in the final pattern.

Because the double segment is at most eight cells wide at the blastoderm stage, all seven of the remaining pair-rule gene products would not be required to specify unique identities to each cell within the double segment. It follows that not all pair-rule genes would play exactly the same part in the process (see below) — yet no one anticipated the surprising result (reported in this issue by Kilchherr *et al.*²) obtained when another pair-rule gene, *paired*, was cloned.

This gene is almost the archetypal pair-rule mutation; the deletion patterns in mutant embryos define seven stripes with the standard double-segment spacing. But analysis of the final pattern of transcripts at late blastoderm revealed fourteen stripes rather than the expected seven, indicating a periodicity corresponding to single-segment primordia. Interestingly, this single-segment mode of expression is

preceded at a slightly earlier stage by seven broad stripes; it is (presumably) the subsequent 'splitting' of these stripes caused by elimination of transcripts from cells in the middle which gives rise to the final pattern. Although the stripes in this transient double-segment mode are too broad to correspond exactly to the more limited deletions observed in *paired* mutants, it is possible that this earlier pattern is the one most relevant to the establishment of the final pattern. In any event, the exact relationship between the relatively simple deletion pattern observed in mutant embryos and the more complicated pattern of transcription remains a mystery.

As we have described above for the *paired* pattern, a static description of the final or most stable pattern of striped transcripts from a given pair-rule gene is likely to yield an incomplete picture of a complex, dynamic process. An understanding of how segments are established and maintained requires an understanding of how the patterns arise, how these transcript patterns relate to translational expression and, ultimately, determination of the functions of the gene products. Ideally, such analyses will be extended to all the segmentation genes; meanwhile, most work has focused on the pair-rule gene *ftz*, and on another gene, *engrailed*, which is required later during development in the posterior compartment of each segment¹⁰, but also reveals a pair-rule phenotype in mutant embryos and is transiently expressed in a double-segment mode during the blastoderm stage.

The establishment of a periodic, segmental pattern along the length of the embryo is thought to be initiated by the zygote in response to some maternally derived factor(s) present in the egg, but the nature of this global mechanism is not understood. One approach to studying this problem has been careful reconstructions of transcript distributions during the stages when the pattern is being formed. In general, transcripts arise in a defined sequence, with stripes in anterior regions becoming apparent before posterior ones. This sequence provides the first indication that the segmental pattern may be established progressively, possibly analogous to the gradual addition of the more posteriorly situated somites observed during vertebrate development. Furthermore, Weir and Kornberg¹¹ show that *engrailed* and *ftz* arise with even broader bands of specificity than the double segment. In pre-cellular stages, the transcripts are spaced at four-segment and even broader intervals, suggesting that the final segment pattern arises by progressive subdivision of larger units into smaller ones.

When fluorescently labelled antisera to the *ftz* and *engrailed* proteins were obtained, it was shown that in those cells which express either gene, the protein is

localized in the nucleus^{12,13}. This presumably reflects the postulated DNA-binding activity conferred by the homoeobox domain present in each protein, and is consistent with a postulated regulatory role in programming cells for specific patterns or fates. The use of antisera has also provided more detailed information on the spatial patterns of gene expression and how they are achieved, while confirming most of the general features inferred from transcript distributions. The distribution observed with the *ftz* antiserum suggests that the final stage of the patterning process involves two clear phases, with the initially broad stripes tightening up into narrower bands as gastrulation begins. This contrasts with the patterning of engrailed stripes, which are generally a single cell wide at the time they arise.

The transition in the blastoderm patterns of *ftz* suggests that establishment of the final pattern of that gene is not a direct response to maternal cytoplasmic cues, but instead involves some kind of fine-tuning among zygotically active loci. This possibility is currently being investigated in several laboratories by analysis of the striping patterns in mutant embryos. Howard and Ingham¹⁴ have recently analysed transcripts of *hairy*, *ftz* and *engrailed* in embryos mutant for either *hairy* or *ftz*. The pattern of *engrailed* expression is altered in either pair-rule mutant, with the usual pattern of fourteen stripes reduced to seven in each case, reflecting the deletion of alternating segmental repeats. These results are gratifying, but not entirely unexpected, because *engrailed* and other genes that are required later in every segment are likely to be regulated in response to the pattern established by the pair-rule genes at the blastoderm stage.

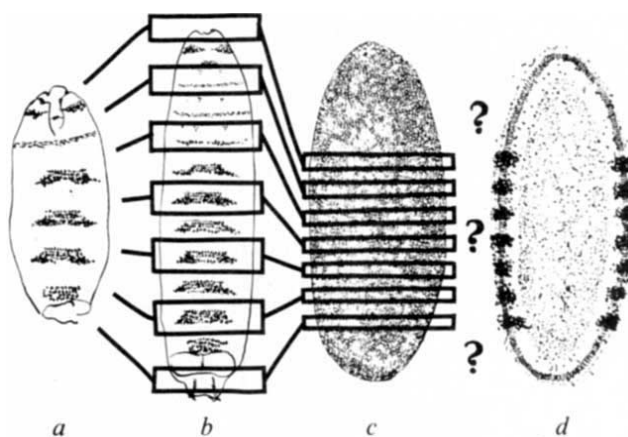
A more surprising result is that the pattern of *ftz* expression is altered by mutations in *hairy*, whereas the *hairy* pattern appears normal in *ftz* blastoderms. Thus, the two pair-rule genes seem to play different parts in the process of segmentation, with *hairy* somehow acting

to regulate *ftz* (and possibly other pair-rule genes). Because the *ftz* stripes become much broader, apparently extending further into the domain where *hairy* is normally expressed, *hairy* might at first glance seem to be a simple repressor of *ftz* transcription; the existence of overlapping domains of transcription observed in wild-type blastoderms (see above), however, suggests that the nature of the interaction is more complex. Also, it is not clear whether the only role of *hairy* is to regulate some subset of the pair-rule genes, or whether it also acts together with these genes to regulate processes further downstream. An understanding of segmentation will require the elucidation of all such hierarchical regulatory relationships, both within each 'class' and be-

morphological landmarks at the stage and the scatter of grains in the emulsion above each stripe. Moreover, the exact deletion domain in a given phenotype is difficult to define because the patterns vary slightly from one mutant individual to the next, even when the genotype is kept constant. But all these inaccuracies are relatively small compared with the differences we outlined earlier in this article, and it is unlikely that the discrepancies will be resolved by more detailed examinations of the phenotype or transcript patterns.

A more likely explanation is that the discrepancy reflects some fundamental difference between molecular and genetic approaches. Transcript analysis determines where RNAs accumulate, independent both of their role in development and

of whether they are ever translated (although experiments with the appropriate antisera should resolve that point). Mutations, on the other hand, will only detect gene products if they are needed. They provide no direct indication of where in the embryo those products are made. These differences in techniques provide two classes of explanation for the discrepancy between molecular and genetic results. In the genetics version, *ftz* and *engrailed*, for example, may be transcribed in broader patterns during pre-blastoderm stages not because those transcripts are required, but because whatever condition controls their expression at the double-segment stage is already present and distributed with a broader spacing at earlier stages. In this view, the early transcripts provide a valuable assay for understanding how *ftz* or *engrailed* transcription is controlled, although the elimination of those transcripts does not block the segmental pattern in the larger



Relationship between segmentation phenotypes and transcription patterns at the blastoderm stage. Embryos homozygous for *ftz* differentiate as larvae with an abnormal segmentation pattern (a) in which the odd-numbered denticle bands have been eliminated (b). The cells in the wild-type embryo that would normally make those pattern elements are located in the blastoderm stage as seven parallel stripes (c), each of which is about four cells wide. When sectioned, blastoderms (d) are hybridized with labelled *ftz* DNA to determine the normal location of *ftz* transcripts, the transcripts also occurring in seven stripes. The positions of the stripes seem to correspond to the locations of those cells which normally make the pattern elements deleted in *ftz* mutant embryos. Recent results suggest that this correspondence is not maintained for other segmentation loci (see text).

tween classes of segmentation genes.

An interesting question which arises from the early *ftz* and *engrailed* transcript patterns (see above) is that the deletions in the corresponding mutant embryos give no clear indication of a requirement at any stage earlier than the double segment. The earlier striping patterns, as well as the localization of *paired* transcripts to fourteen rather than seven stripes, argue that the relationship between phenotypes and transcript distributions must be re-examined. How can such discrepancies be resolved? In some cases, it could be argued that the differences observed are artefactual; or that the patterns of transcription at the blastoderm stage can never be described with absolute accuracy within the eight-celled double-segment interval because of the scarcity of gross

(earlier) modes and the early transcripts have no obvious function. Similarly, one could assume that only every other stripe of transcripts observed in the fourteen-stripe *paired* pattern is required, or that only the earlier, seven-stripe mode is key to the functioning of the gene.

Alternatively, in the molecular view, gene products are made only in the cells where they are required. In this explanation, the molecular patterns at the blastoderm stage would give the best indication of the actual role of the gene product during development. Unexpected features of the phenotype of mutant embryos could be explained as secondary interactions between the cells once an abnormal pattern has been established at the blastoderm stage. Explanations of this kind demand that mutant phenotypes be

1. Nüsslein-Volhard, C. & Wieschaus, E. *Nature* **287**, 795 (1980).
2. Kilchherr, F. *et al.* *Nature* **321**, 493 (1986).
3. Weiner, A. *et al.* *Cell* **37**, (1984).
4. Kuroiwa, A. *et al.* *Cell* **37**, 825 (1985).
5. Hafen, E. *et al.* *Cell* **37**, 833 (1984).
6. Garcia Belido, A. *Ciba Fdn Symp.* **29**, 161 (1975).
7. Gergen, J.P. *et al.* *Symp. Soc. dev. Biol.* (in the press).
8. Crick, F. & Lawrence, P. *Science* **189**, (1975).
9. Ingham, P.W. *et al.* *Nature* **318**, 439 (1985).
10. Kornberg, T. *et al.* *Cell* **40**, 45, (1985).
11. Weir, M.P. & Kornberg, T. *Nature* **318**, 433 (1985).
12. Carroll, S.B. & Scott M.P. *Cell* **43**, 47 (1985).
13. DiNardo, S. *et al.* *Cell* **43**, 59 (1986).
14. Howard, K. & Ingham, P. *Cell* **44**, 949 (1986).
15. Gergen, J.P. & Wieschaus, E. *Dev. Biol.* **109**, 321 (1985).

at least partially non-autonomous in genetic mosaics.

Although large-scale non-autonomy has not been observed in the analysis of segmentation phenotypes, the sample of loci tested so far is relatively small¹⁵. Also, it is not clear whether the techniques presently available for marking cells in *Drosophila* are sufficiently accurate to exclude the limited non-autonomy needed to explain the discrepancy between the phenotypes and transcript distributions.

However, genetic mosaics ultimately provide the most direct technique for relating phenotypes to individual cell requirements, and the variety of mosaic techniques available in *Drosophila* should provide a better understanding of the relationship between the gene products and the patterning process. □

Douglas Coulter and Eric Wieschaus are in the Department of Biology, Princeton University, Princeton, New Jersey 08544, USA.

Mathematics

The class number problem

from Ian Stewart

SOME of the deepest mathematical theories have been built from very humble raw materials, and a classic case is number theory, which studies properties of ordinary whole numbers 1, 2, 3, ... It has long been remarked that, although it is easy to observe patterns in the behaviour of whole numbers, it can be astonishingly hard to prove that these patterns are generally valid. Number theorists have long been interested in the arithmetic of 'imaginary quadratic fields', systems of numbers in the form $x + y\sqrt{-D}$, where D is a whole number and x, y are rational.

Any whole number can be factorized into primes, and there is an analogous concept for such fields. For whole numbers, the prime factorization can be achieved in only one way. This is not always true for imaginary quadratic fields; and in his *Disquisitiones Arithmeticae* of 1801, the fount of all modern number theory, Carl Friedrich Gauss in effect conjectured that unique factorization holds precisely when $D = 1, 2, 3, 11, 19, 43, 67$ and 163. This conjecture was proved more than a hundred years later by A. Baker (*Mathematika* **13**, 201; 1966) and independently by H.M. Stark (*Michigan Math. J.* **14**, 1; 1967). But Gauss also stated a more general conjecture, the class number problem, the solution of which by D.M. Goldfeld, B. Gross and D. Zagier has recently been described in a masterly survey by Goldfeld (*Bull. Am. Math. Soc.* **13**, 23; 1985).

The classic example of non-unique factorization occurs in the case $D = 5$. Here $6 = 2 \cdot 3 = (1 + \sqrt{-5})(1 - \sqrt{-5})$ exhibits two distinct prime factorizations. (Each of the numbers 2, 3, $1 + \sqrt{-5}$ and $1 - \sqrt{-5}$ is prime in this particular number field.) The extent to which factorization is not unique can be measured by a quantity called the class number, h : when $h = 1$, factorization is unique, and for larger values of h it is not unique. In some sense the larger h becomes the less unique factorization is. For a given D it is relatively easy to calculate the class num-

ber (for example, if $D = 5$ then $h = 2$) but the list of values so obtained varies in a very irregular way with D . This makes it hard to solve the inverse problem: given a value for h , find which D s have that class number. And this is what Gauss's conjectures are about. The conjecture on unique factorization asks for a determination of all D such that the class number is 1, with the guess that they are precisely the 9 values listed above. The general class number problem is to prove that for any given class number h , the list of D having that value for h is finite.

Gauss actually phrased his conjectures in a slightly different setting, the theory of quadratic forms. This goes back to the time of Pierre de Fermat, who stated theorems of the following kind: "Every prime of the form $6n + 1$ can be written as $x^2 + 3y^2$ for integers x and y ." For example, 31 is such a prime, and $31 = 1^2 + 3 \cdot 3^2$. The connection with quadratic fields is that $x^2 + 3y^2 = (x + y\sqrt{-3})(x - y\sqrt{-3})$, a factorization in the field corresponding to $D = 3$. So theorems about quadratic fields carry implications for quadratic forms, and conversely.

Gauss, and Joseph-Louis Lagrange before him, realized that it is not necessary to study all possible quadratic forms, because changes of variable can be used to turn one form into another. For example, if x and y are replaced by $x + 2y$ and $x + y$, respectively, then the form $x^2 + 3y^2$ becomes $4x^2 + 10xy + 7y^2$. Therefore, the numbers that can be represented in the form $x^2 + 3y^2$ are precisely those that can be represented in the form $4x^2 + 10xy + 7y^2$, even though at first sight these are different questions. This led Gauss to the idea of 'equivalent' forms (transformable into each other by such changes of variable). He proved that there is only a finite number of distinct classes of equivalent forms, which is the class number of the associated quadratic field.

H. Heilbronn and E.H. Linfoot (*Q. J. Math.* **5**, 293; 1934) showed that, apart from the nine known cases, at most one

further field has class number 1. In 1952 Kurt Heegner claimed a proof that no such tenth field exists, which would solve the class number 1 problem. But as Goldfeld remarks: "Heegner's paper contained some mistakes and was generally discounted at the time. He died before anyone really understood what he had done."

Baker's proof uses new results from the theory of transcendental numbers (numbers that do not satisfy any polynomial equation with rational coefficients), whereas Stark's proof is much closer in spirit to Heegner's attempt. Then M. Deuring (*Inventiones Mathematicae* **5**, 169; 1968) showed that the 'gap' in Heegner's attempted proof can be filled relatively painlessly. In 1971 Baker (*Ann. Math.* **94**, 139) and Stark (*Ann. Math.* **94**, 153), again independently, solved the class number 2 problem, finding exactly 18 values of D for which $h = 2$.

Although these results represent enormous progress compared with what was previously known, it is clearly not feasible to tackle the general problem one class number at a time, because the process will never end. The interest of such partial answers is that they suggest new methods. Goldfeld (*Astérisque* **41-42**, 219; 1976) exploited a connection between the class number problem and an elliptic curve, a type of cubic equation. He showed that if just one elliptic curve can be found with a particular property, then the class number problem is solved. Unfortunately, no such elliptic curve was then known. But later, Gross and Zagier (*C.r. hebdomadaire. Séances Acad. Sci., Paris* **297**, 85; 1983) established the necessary property for the elliptic curve $-139y^2 = x^3 + 4x^2 - 48x + 80$. By such roundabout methods was Gauss's conjecture on the finiteness of the list of imaginary quadratic fields of given class number demonstrated.

Soon afterwards, J. Oesterlé (*Séminaire Nicolas Bourbaki* 631; 1984) obtained a specific estimate for the size of the largest D with given class number h , making it possible in principle to select values of h and find all possible D s. As a result, the class number 3 problem (precisely which D gives $h = 3$?) has been solved, and many long-standing problems in number theory begin to appear tractable. For example, a solution of the class number 4 problem would answer a famous question: which integers can be expressed as a sum of three squares in exactly one way? The powerful and beautiful ideas laid bare by a 180-year assault on Gauss's conjecture strikingly attest to the quality of his insight into the deep properties of ordinary whole numbers. But the sad story of Kurt Heegner shows that genius is still not always appreciated during its lifetime. □

Ian Stewart is at the Mathematics Institute, University of Warwick, Coventry CV4 7AL, UK.

Evolution — the struggle continues

SIR—In an interesting and provocative article, Halstead¹ rejected the anti-darwinian views of the Japanese biologist Imanishi. His rejection is apparently based on his negative impressions of Japanese society. Halstead remarked that: "the ordinary Japanese [is] condemned... to the rigid authoritarian feudal society that masquerades as one of the advanced nations of the world."

Halstead sees the world as "demonstrably... [one]... of competition, struggle and disharmony". This is not surprising when one considers that the concept of competition is inherent to Neo-Darwinism and that Halstead is a staunch supporter of this theory. However, while Halstead may see the world in this way, the question remains — is there any evidence for the reality of what he sees?

Halstead states: "Recent research... [has]... demonstrated that interspecific competition takes place in 90 per cent of all cases studied". On the contrary, a critical review of a large number of cases by Underwood² suggests that this statement is grossly inaccurate. This also suggests that Halstead's claim that "the informational foundation of Imanishi's theory no longer stands" is incorrect. Irrespective of this, the conclusive demonstration of competition is unlikely to deter the Japanese public from supporting Imanishi's theory. As suggested by Brady³, there may be more than one reason for accepting a theory. This is not an attribute unique to the Japanese scientific community, as suggested by Halstead, but a characteristic of the development of theories in general.

We agree with Imanishi that the selectionism of Darwin has its roots in Western society. In the same way Imanishi's theory has been influenced by Japanese society. However, while the theory was developed by a Japanese, it is not necessarily uniquely Japanese in concept and therefore judgement of the theory on the basis of the values of the society in which it was developed cannot be feasible. After reading Halstead's article, one is left with the distinct impression that this is exactly what he has done.

C. D. MILLAR
N. R. PHILLIPS
D. M. LAMBERT

*Evolutionary Genetics Laboratory,
Department of Zoology,
University of Auckland,
Private Bag, Auckland, New Zealand*

1. Halstead, B. *Nature* **317**, 587-589 (1985).

2. Underwood, A.J., Anderson, D.J. & Kikkaw, J. (eds) *Community Ecology: Pattern and Process* (Blackwell, Oxford, 1984).

3. Brady, R.H. *Biol. J. Linn. Soc.* **17**, 79 (1982).

SIR—Halstead's commentary "Anti-

darwinian theory in Japan" (*Nature* **317**, 587; 1985) introduces the theory of evolution advanced by Imanishi, at the same time rejecting it. We feel, however, that Halstead rejected Imanishi's theory of evolution without fully understanding it, and we wish to comment on the theory on purely scientific grounds.

First, Halstead introduces Imanishi's concept of habitat segregation and comments that this is but a "dream" attractive to members of Japanese society, which is actually extremely competitive. Leaving aside the rather meaningless discussion as to whether or not Japanese society is more competitive than that of Europe or America, we would like to know whether Halstead will recognize habitat segregation as a principle on which a wholly different theory of evolution could be built. We consider habitat segregation to be a real biological phenomenon.

Second, if Halstead recognizes habitat segregation as propounded by Imanishi as a possibility, he ought either by explaining habitat segregation by means of Darwinism, include it in Darwinism or clearly recognize Imanishi-ism as a view of evolution which is independent of Darwinism. Interestingly, Halstead does not attempt this but tries to give a "sociological" explanation of Imanishi's theory. We think that there is a relationship between the competitive exclusion principle of Gause¹ and Hardin² and Imanishi's concept of habitat segregation.

Third, Imanishi strongly refutes Darwin's hypothesis of the survival of the fittest and asserts that: "Survival is in most cases purely accidental. It is a matter of luck rather than selection". Darwin considers the struggle for existence to be fierce among individuals of the same species as well as between different species, but Imanishi is critical of this idea. Imanishi, as Halstead stresses several times, understands evolution as being a transformation only occurring to the species, and not to the individual. In contrast to Darwinism, which considers the individual to be the unit of evolution and expends great energy in explaining how the transformations of this individual are magnified in the transformations of the species, Imanishi asserts that the unit for explaining evolution is not the individual but the species.

We believe this idea of the species as a unit to be the essence of Imanishi's assertion that "all the individuals of a species change at once when the time to change comes" will be understood. Concerning this unit of evolution, we support Imanishi's ideas. In the case of the law of movement of heavenly bodies, the Earth and the Moon are considered the units regardless of their components. The minute examination of brain cells probably will not be able to tell us how humans

think. Building theories to explain natural phenomena requires an appropriate unit. The originality of Imanishi's theory of evolution is the idea of the species as its unit. We would like to ask what Halstead's opinions are upon this point.

We consider that Darwinism and Imanishi's theories are two separate views of evolution. If Halstead feels that Imanishi's theory of evolution is unscientific, that it could not even stand as a hypothesis, he should give scientific reasons, and not literary or sociological explanations.

HIDEOMI NAKAHARA
*Department of Environmental Health,
Medical University of Yamanashi,
Yamanashi, Japan*

TAKASHI SAGAWA
TAKASHI FUKU
*Institute of Human Sciences,
Tokyo, Japan*

1. Gause, G.F. *The Struggle for Existence* (Hafner, 1934).
2. Hardin, G. *Science* **131**, 1292-1297 (1960).

Kenyan finds not early Miocene *Sivapithecus*

SIR—In his recent commentary in *News and Views* questioning the assignment of the new Miocene hominoid specimens from Buluk, Kenya¹ to *Sivapithecus*, Delson² referred to a paper of ours in which we discuss the hominoid genus *Ouranopithecus* from Ravin de la Pluie, Greece³. As he states, we suggested a link with "hominines", but one based upon a particular assumption about character state polarities drawn from several alternatives, any of which, as we stated, might be correct.

He failed to note another argument in that paper which goes to the heart of the issue of the generic status of the Buluk specimens. As others have done, we argue for a *Sivapithecus*-orangutan clade based on a set of facial characters worked out by Ward⁴ and Andrews⁵ relating to the premaxilla and palate. As the clade is defined by these presumed shared derived features, any species included in *Sivapithecus* must also possess these character states. By these criteria, only *Sivapithecus* species from the Siwaliks of Indo-Pakistan and *S. metei* from the Sinap series in Turkey are definitely included in *Sivapithecus*. *Ouranopithecus* from Ravin de la Pluie^{3,6}, *Rudapithecus* or *Dryopithecus* from Rudabanya, Hungary⁷, *Kenyapithecus* from Nachola, Kenya⁸, and the large hominoid from Lufeng, China (personal observation) clearly are not. The status of other thick-enamelled hominoid samples which lack specimens with the critical nasopalatal morphology, such as that from Buluk, must remain open, although in some other features Buluk specimens differ markedly from Asian *Sivapithecus*.

We have provided a specific diagnosis

of *Sivapithecus* based upon a set of character states that most now accept as being derived features showing phyletic affinity with the orangutan. It is a framework in which neither the definition nor the species inclusivity of *Sivapithecus* is as ambiguous as Delson suggests.

JAY KELLEY

Division of Biology and Medicine,
Brown University,
Providence,
Rhode Island 02912, USA

DAVID PILBEAM

Department of Anthropology,
Harvard University, Cambridge,
Massachusetts 02138, USA

1. Leakey, R.E.F. & Walker, A. *Nature* **318**, 173-175 (1985).
2. Delson, E. *Nature* **318**, 107-108 (1985).
3. Kelley, J. & Pilbeam, D. *Am. J. phys. Anthropol.* **66**, 188 (1985).
4. Ward, S.C. & Kimbel, W.H. *Am. J. phys. Anthropol.* **61**, 157-171 (1983).
5. Andrews, P. & Cronin, J.E. *Nature* **297**, 541-546 (1982).
6. de Bonis, L. & Melentis, J. *C. r. hebdo. Séanc. Acad. Sci. Paris* **300**, 429-432 (1985).
7. Kelley, J. & Pilbeam, D. in *Comparative Primate Biology* Vol. 1 (ed Swindler, D.R.) (Liss, New York, in the press).
8. Pickford, M. in *Hominid Evolution: Past, Present and Future* (ed. Tobias, P.V.) 107-112 (Liss, New York, 1985).

Quantum behaviour of superconducting rings

SIR—In a recent *Nature* (**319**, 726; 1986), U. Eckern discussed the applicability of quantum mechanics to macroscopic objects, for which superconducting rings incorporating weak link constrictions are considered to be very strong candidates. To set this in context, we wish to point out that there are modes of operation of weak link rings other than the one considered by Eckern. For these, experimental results already exist which are very strong evidence for the fact that such rings do indeed behave as macroscopic quantum objects.

The magnetic flux threading the superconducting ring ϕ and the electric charge localized at the weak link Q are the conjugate variables for a weak link ring. If it is a quantum object a Heisenberg relation for the flux and charge uncertainties $\Delta\phi\Delta Q \geq \hbar/2$ exists. Eckern addressed only the limit where the flux is exceedingly well defined compared to the quantum unit of flux ϕ_0 ($\phi_0 = \hbar/2e$ where $\hbar$ is Planck's constant and e the electric charge), so $\Delta\phi \ll \phi_0$, and thus $\Delta Q \gg 2e$, $2e$ being the charge on a Cooper pair of electrons in the superconductor. Our experimental and theoretical work over the past few years has considered two other limits: (1) where the flux is quite well defined $\Delta\phi \ll \phi_0$, and the charge is fairly uncertain $\Delta Q \gg 2e$. (2) Where the charge is quite well defined $\Delta Q \ll 2e$, and the flux is fairly uncertain $\Delta\phi \gg \phi_0$. We have accumulated a large amount of experimental data which are in accord with the predictions made theoretically by considering weak link rings as quantum objects, and which to date have no alternative explanation.

Readers who are interested in the quantum mechanics of macroscopic objects but are unfamiliar with the published literature on superconducting weak link rings are referred, for example, to *Helv. phys. Acta* **56**, 789 (1983) and *Phys. Lett.* **104**, 375 (1984); **107A**, 133 (1985); **111A**, 199 (1985); **115A**, 125 (1986).

TIMOTHY P. SPILLER
T.D. CLARK

Physics Division,
School of Mathematical and
Physical Sciences,
University of Sussex,
Brighton BN1 9QH, UK

The stability of zoological nomenclature

SIR—The long letter on this subject by Erzinclioglu and Unwin (*Nature* **320**, 687; 1986) reveals some fundamental misunderstandings. But if they, or others prompted by their letter, will make constructive suggestions then a valuable purpose will have been served.

As stated in its introduction, the *International Code for Zoological Nomenclature* (3rd Edn, 1985) "provides guidance for zoologists needing to establish new names, and rules to determine whether any name, previously proposed, is available and with what priority..." It is a do-it-yourself manual and like any must be used with commonsense for the desired results, in this case stability coupled with taxonomic freedom.

The purpose of the International Commission on Zoological Nomenclature is the very opposite of that portrayed in the situation graphically described by Erzinclioglu and Unwin: "taxonomists... are kept in place by the tyranny of the Commission: the Code must be obeyed". Their statements that "[in some circumstances] a name must still be changed in spite of the confusion that will be caused" and "there is only one course open to us if sanity is to be restored and that is that some sections of the code must be consistently ignored" are not accurate and overlook the basic function of the Commission.

Some code of zoological nomenclature, internationally recognized, must clearly exist, and like any other set of 'rules' the existing one is imperfect and could not be otherwise. The number of zoological names runs into millions, and naturally rigid adherence to the code's prescriptions will sometimes cause confusion. Individual zoologists (in the widest sense of the word) cannot follow the recommendation of Erzinclioglu and Unwin and "consistently ignore some sections of the Code", because in the nature of things they would be inconsistent. The International Commission, at present of 25 scientists from 15 countries, exists largely to overcome this problem.

Anyone encountering a difficulty, for example a conflict between the priority of one name and the established usage of another (the blood-sucking maggot mentioned by Erzinclioglu and Unwin is a case), should submit the matter to the Commission. This does not then act in any tyrannical manner, but solicits the comments of zoologists by publishing the problem in the quarterly *Bulletin of Zoological Nomenclature*, and also by notifying appropriate journals. The Code (Article 80) provides that existing usage should be maintained meanwhile. Comments adding any new facts are published in the *Bulletin*, and in every case are brought to the attention of the Commission. After at least six months (commonly two years, although this is being reduced) the members of the Commission vote by postal ballot, and a two-thirds majority will suffice to set aside the provisions of the Code if appropriate. The outcome is published as an 'Opinion' in the *Bulletin*. Of course there is no power to enforce anybody either to approach the Commission or to abide by its conclusions, but it is in the common interest that they should do so.

The 1985 Code is a carefully considered result of very numerous contributed suggestions, although none were proposed as "devices to ensure unnecessary name changes"! Of course it needs amendments. One is proposed by Erzinclioglu and Unwin (although they are not the first), and concerns the requirement that the gender of generic and specific names should agree. This was clear in the past when naturalists knew Latin and classical Greek, but many feel it unsuited to the present day of computer information retrieval (see p.xix of the Code's introduction). The Commission invites views on this and on any matters concerning zoological nomenclature.

P.K. TUBBS

International Commission for
Zoological Nomenclature,
c/o British Museum (Natural History),
London SW7 5BD, UK

In the eye of the beholder

SIR—T. Nash (*Nature* **320**, 402; 1986) believes that the patches he sees in the out-of-focus image of a light point originate from individual light receptors in his retina. If he set about measuring their angular subtense, he would discover that they are about an order of magnitude larger than those of retinal cones. And how does he think he gets to "see" the spaces between the "receptors"? In fact, Nash is viewing entoptically the quality of his eye's optics and the transparency of his crystalline lenses.

GERALD WESTHEIMER

University of California,
Berkeley, California 94720, USA

Error rates in prenatal cystic fibrosis diagnosis

SIR—Pembrey and Malcolm¹ state that if a prenatal diagnosis is based on an empirical correlation between an amniotic fluid protein and fetal genotype, an attempt should be made to confirm diagnosis on the abortus after termination of pregnancy. This is certainly standard practice for α -fetoprotein-based diagnosis of neural tube defects, and has become part of our procedure for microvillar enzyme-based prenatal diagnosis of cystic fibrosis². Apart from providing a continual updating of the sensitivity and specificity of the test, such procedures are useful for monitoring the inevitable errors of sample handling which creep into even the best-regulated laboratories.

What puzzles us is why Pembrey and Malcolm should feel that diagnosis based on linked DNA probes should be excluded from such quality control. The accuracy of such diagnosis is dependent on recombination frequencies. The derivation of recombination frequencies is a form of empirical correlation and only as good as the most recent set of data. In fact, because the total number of informative meioses observable can be quite small, recombination fractions may be subject to dramatic fluctuations. The linkage between the G8 probe and the Huntington's disease gene was initially very tight³, but recombinants appeared with the acquisition of new family data^{4,5}. The same is true for linkage between the cystic fibrosis gene and the probes, pJ3.11 and met^{6,7}. Recombination fractions are usually stated as lying within a given confidence interval, to indicate the uncertainties inherent in their estimation.

There is also the question of genetic heterogeneity. Pembrey and Malcolm suggest that careful linkage studies in a large number of families from different populations will allow exclusion of significant non-allelic heterogeneity. But what do they mean by significant? An average non-allelic heterogeneity level of 5 per cent would be very significant if it meant that a similar proportion of prenatal diagnoses were wrong. It is difficult to see how this figure can go much below 5 per cent when the error rate in diagnosing cystic fibrosis is believed to be of this order⁸.

The point that we were making in our earlier letter⁹ is that the whole edifice of quality control is not based on pedantry but on the realization that laboratories do make mistakes. It is possible that, as first-trimester prenatal diagnosis becomes more widely available, the standard procedure of confirmation of diagnosis on abortuses will have to be abandoned. We should, nonetheless, continue to collect and analyse the outcomes of as many of these pregnancies as is possible, since it will improve the reliability of the linkage

data. And let us be clear what we are doing, and not imply — as do Pembrey and Malcolm — that we have acquired new procedures which are now error-free.

DAVID J.H. BROCK

Human Genetics Unit,
University of Edinburgh,
Edinburgh, UK

VERONICA VAN HEYNINGEN

MRC Clinical and Population
Cytogenetics Unit,
Western General Hospital,
Edinburgh EH4 2XU, UK

1. Pembrey, M.E. & Malcolm, S. *Nature* **320**, 114 (1986).
2. Brock, D.J.H. *et al. Lancet* **i**, 1175 (1985).
3. Gusella, J.F. *et al. Nature* **306**, 234 (1983).
4. Folstein, S.E. *et al. Science* **229**, 776 (1985).
5. Youngman, S. *et al. J. med. Genet.* **21**, 299 (1984).
6. White, R. *et al. Nature* **318**, 382 (1985).
7. Wainwright, B. *et al. Nature* **318**, 384 (1985).
8. Denning, C. *et al. Pediatrics* **66**, 752 (1980).
9. Brock, D.J.H. & van Heyningen, V. *Nature* **319**, 184 (1986).

Homology of trichosanthin and ricin A chain

SIR—Trichosanthin is a plant protein extracted from a Chinese herbal medicine, the root tuber of *Trichosanthes kirilowii maxim*, Cucurbitaceae. It has been shown to bind to the trophoblastic syncytial layer killing the cells quite selectively. It can therefore be used clinically to terminate pregnancy and inhibit trophoblastic tumours¹. This basic single-chain protein consists of 234 amino acid residues. Its sequencing has recently been completed^{2,3} and a molecular model has been derived from the X-ray analysis⁴. Here we report a striking degree of sequence homology between trichosanthin and subunit A of ricin-D (ricin A for short). It is the first example of the complete sequence comparison between a ribosome-inactivating protein and cytotoxin.

In recent years, much attention has been paid to cytotoxins and the like for theoretical reason and because of their potential as immunotoxins to treat cancers. Many cytotoxins consist of two kinds of subunits. The toxic action is associated exclusively with subunit A which enzymatically inhibits protein synthesis of eukaryotes, whereas one or more B subunits have the ability to bind to cell surfaces and aid subunit A penetration into

cytosol. Among others, typical protein cytotoxins are diphtheria toxin, cholera toxin (from bacteria), ricin and abrin (from plants). However, it has also been found that many plants contain single chain proteins which are very similar to the toxic subunit A of the above cytotoxins in having the ability to inhibit protein synthesis. Pokeweed antiviral protein (PAP), momorcharin, wheat germ inhibitor and gelonin are good examples (for review see ref 5). *Momordica* and *Trichosanthes* are taxonomically closely related and all belong to the Cucurbitaceae family. It has been reported that momorcharin and trichosanthin have similar biological functions⁶. It led us to think that trichosanthin may also fall into the same group. To test for this possible relatedness, we therefore carried out sequence comparison studies. Sequence comparisons between short pieces of N-terminal segments of some ribosome-inhibiting proteins (RIPs) and cytotoxins have been recently reported^{7,8}. Here we report the complete sequence comparison by computer analysis between trichosanthin and ricin A.

Figure 1 demonstrates our alignment of trichosanthin and ricin A, in which 91 residues are identical and 42 conservative. In our analysis, substitutions are taken as conservative only if two conditions are met — a minimum base change of one and that the two side-chain's physico-chemical properties are similar, as with Ser and Thr or Asp and Glu. The identical and conservative residues comprise at least 56 per cent of the trichosanthin sequence.

Further inspection shows that the identical and conservative residues are rather concentrated and that very little deletion needs to be assumed. In particular, there is no deletion at all from residues 97 to 149 in trichosanthin. We therefore believe that the sequence similarity between trichosanthin and ricin A is by no means a random event. It has been reinforced by the probability estimation of the alignment.

It is reportedly very rare for an identical pentapeptide to appear in different chains⁹, the probability is estimated to be about 1×10^{-6} . One may then expect the

1'	I F P K Q Y P	D V S F R L S G A T	S S S Y G V F I S N Q R K A L P M E R K L - Y
1"	I L I N F T T A G A T	V Q S Y T N F I R A V R G R L T T G A D V R H	
33'	D T P L I T - - R S S L P G S Q R V A I T H L T N Y A D - - E V A I D V T N V D		
41"	D T P V L P N R V G L P I N Q R F I L V E L Q N H A E L S V T L A L S V T N A -		
68'	A G L P R N A V L Y I M G Y R A G D T S Y F F - - N E A S A T E A A K Y V F K		
80"	- - - - - Y V V G Y R A G N S A Y F F H P D N Q E D A - E A I T H L F T		
105'	D A M R K V T L F P Y S G N Y E R L Q T A A G G L R E N I P L G L P A L D S A I T		
110"	D V R Y N T F A F G G N Y D R L E Q L A G N L R E N I L G N G P L E E A I S		
145'	T D P Y Y - - - N A N S A A S A L M V L I Q S T S E A A R Y K F I E Q Q T G		
150"	A L Y Y S T G G T Q L P T L A R S F I I C I Q M I S E A A R F Q Y I E G E M R		
180'	S R V D K T - - - F L P S L A I I T L E N S L V L A L S K Q I Q I A S T N N G		
190"	T R I R Y N T R R S A P D P S - - V I T L E N S - V G R L S T A I Q - - E S N Q G		
216'	T F E S P V V L I N A Q N Q R N N H A		
225"	A F A S P I - - - Q L Q R D G S K F S V Y D V S I L L P I I A M V Y R C A P		
260"	P P S S Q F		

frequency of appearance of any identical pentapeptide in both trichosanthin and ricin A to be only about 0.05. In fact, three identical pentapeptides have been found. One is GYRAG in residues 80–84 of trichosanthin and 83–87 of ricin A. The second is LRENI in residues 128–132 of trichosanthin and 133–137 of ricin A. And the third is SEAAR in residues 166–170 of trichosanthin and 176–180 of ricin A. Thus the remarkable sequence homology between trichosanthin and ricin A seems not to be coincidental, and may well be of biological significance.

There is a pool of evidence indicating that it is an enzymatic process by which the toxic subunits of cytotoxins affect the host cell metabolism. For diphtheria toxin and cholera toxin, the subunit A acts on the elongation factor EF2 and GTP-binding protein respectively. Nevertheless they share a common mechanism — ADP=ribosylation. For plant toxins such as ricin and abrin, in spite of considerable efforts, the nature of their enzymatic activity is obscure. It is known, however, that they inactivate the 60S subunit of ribosomes (for review see ref. 5). It is likely that different cytotoxins may not use the same mechanism, but the sequence homology between trichosanthin and ricin A shown here strongly suggests that these two do act by similar mechanisms. Taxonomically, *Ricinus* belongs to the Dicotyledoneae Archichlamydeae Euphorbiaceae *Ricinus* L. and *Trichosanthes* belongs to the Dicotyledoneae Sympetalae Cucurbitaceae *Trichosanthes* L. They are not closely related. But the possibility can not be ruled out that they originate from a common ancestor.

There is a speculation⁷ that in the ancient plants there was a special defensive protein which could inactivate eukaryotic ribosomes. In the evolution process, the gene coding for this toxic chain might have fused with the gene for some sort of sugar-binding proteins, resulting in cytotoxins with two different types of subunits as found in species like *Ricinus*. However, in some plants, such as pokeweed, the toxic chain remains independent and has been termed the ribosome-inactivating protein (RIP). The overall sequence homology between trichosanthin and ricin A strongly supports this hypothesis.

We thank Professor Wang Yu and his colleagues for their kindness in showing us the sequence of tricosanthin before publication. This work was supported by a special grant from the State Commission of Science and Technology of China.

ZHANG XUEJUN
WANG JIAHUI

Biophysics Institute,
Academia Sinica,
Beijing, China

3. Wang Yu *et al.* (International Symposium on Organic Chemistry of Medicinal Natural Products) at Shanghai, China, November 1985.
4. Pan Kezhen *et al.* Supplement of Proc. of China-Japan Bilateral symposium on Biophysics, May, 1985, Wuxi, China.
5. Olsnes, S. & Pihl, A. in *Molecular Action of Toxins and Viruses*, 51–105 (Elsevier, Amsterdam, 1982).
6. Chan, W. Y., Tam, P. P. & Yeung, H. W. *Contraception* **29** (1), 91–100 (1984).
7. Ready, M., Wilson, K., Piatak, M. & Robertus, J. D. *J. biol. Chem.* **259** (24), 15252–15256 (1984).
8. Lappi, D. A., Esch, F. S., Barbieri, L., Stirpe, F. & Soria M. *Biochem. biophys. Res. Comm.* **129**, 934–942 (1985).
9. Kabsh, W. & Sander, C. *Proc. natn. Acad. Sci. U.S.A.* **81**, 1075–1078 (1984).

Putting a charge on a quark

SIR—The magnitudes of the quark electric charges raise the perplexing question: what is the magnitude of the unit electric charge? When the proton and electron were thought to be the elementary particles of nature, it appeared quite acceptable to take the electronic charge as the unit and to express all other charges in terms of it but, if quarks are elementary particles, it is meaningless to speak of a unit charge in any unambiguous way; only charge ratios are meaningful.

It is thus sensible to ask why the charge on the *u* (up) quark is minus twice that on the *d* (down) quark and why the latter is one third that of the electron, but it is not meaningful to ask which of these is the unit charge. If it is desirable to work with integral charges, the *d*-charge should be taken as the unit; the *u*-charge is then -2 units and the electronic charge is $+3$ units. That the latter is universally accepted as the unit charge is merely a matter of a bias which perpetuates the belief that the electron is an elementary particle for which, at present there is no conclusive evidence.

Nothing in current particle theories even begins to answer the question why only two elementary charges exist in nature and why their ratio is -2 . Nor can these theories indicate how the answer is to be found. Quantum chromodynamics confuses things even more for it raises the additional question as to why all colours of a given quark flavour have the same electric charge.

If the three quarks in a nucleon form a linear structure, with one of the quarks always at the centre, as I have already proposed in my gravitational linear rotator model of the nucleon, then a simple variational principle answers the questions about quark charges.

In a series of papers I have proposed a linear rotator model of baryons in which the constituent particle triplets are gravitationally bound unitons, particles of mass $m \approx (\hbar c/G)^{1/2}$, where G is the universal gravitational constant. The gravitational binding force between any two such particles is thus larger than the electrostatic force by a factor of the order of 137. Accepting this model one sees that the overall dynamical configuration is determined essentially by the gravitational field

and that the electromagnetic field plays only a minor role in it; that is, the linear arrangement of three unitons, with one at the centre, is a consequence of the large masses of the unitons and would persist if the three unitons were electrically neutral. Nevertheless, the assignment of electric charges to the unitons does matter, for only one such assignment among the various possibilities is preferred by the energetics of the problem.

I now show that one obtains this unique uniton charge distribution by imposing the condition that the electrostatic potential energy of the linear triplet be a minimum. I have assumed here that gravity is the dominant force in this linear nucleon model, but any strong force field that gives the same linear arrangement will do.

If Q is the charge on the nucleon and q_1, q_2, q_3 , are the three quark charges, we have:

$$q_1 + q_2 + q_3 = Q \quad (1)$$

where q may be positive or negative. Placing q_1 at the centre of the linear configuration and q_2 and q_3 at the ends, the electrostatic potential energy is:

$$E = \frac{1}{r}(q_1 q_2 + q_1 q_3 + \frac{1}{2} q_2 q_3) \quad (2)$$

where $2r$ is the length of the structure; or,

$$rE = q_1(q_2 + q_3) + \frac{1}{2}(q_2 q_3) \\ = q_1 Q - q_1^2 + \frac{1}{2} Q q_2 - \frac{1}{2} q_1 q_2 - \frac{1}{2} q_2^2 \quad (3)$$

where I have used (1) to eliminate q_3 . Varying this with respect to q_2 , keeping Q and q_1 fixed, leads to the condition that $d(rE)/dq_2 = 0$ if, and only if, $q_2 = q_1$.

In other words, regardless of the value of Q and which uniton is at the centre of the configuration, the charges on the two ends must be equal in magnitude and in sign. Moreover, from (1), $q_2 = \frac{1}{2}(Q - q_1)$ or $q_2 = \frac{1}{2}(1 - q_1)$ if Q is the unit charge of the proton.

If, now, quark q_2 is at the mid point of this configuration, $q_2 + 2q_1 = Q'$, where Q' is another value of the total charge, to be identified with that of the neutron, so that $q_2 = -2q_1$. But from the equivalent information for the proton $-2q_1 = \frac{1}{2}(1 - q_1)$, or $(1 + 3q_1) = 0$ or $q_1 = -1/3$ hence $q_2 = 2/3 q_1$.

This is as far as one can go with this simple type of analysis, but its simplicity and its seeming meagreness hide its importance, for it clearly points to the linear rotatore with a uniton (quark) at its centre as the correct model of the nucleon. We are ineluctably led to this conclusion by the coefficient $-1/2$ in front of q_2 in equation (3); if it were numerically different, we would not obtain the important equality $q_2 = q_3$.

LLOYD MOTZ

Department of Astronomy,
Columbia University,
New York 10027, USA

1. *Acta zoologica sinica* (Chinese) **22**, 126–136 (1974).

2. Gu Ziwei *et al.* *Acta chemica sinica* (Chinese) **43**, 943–945 (1984).

Consent — or compulsion?

Eric Ashby

National Styles of Regulation: Environmental Policy in Great Britain and the United States. By David Vogel.

Cornell University Press: 1986. Pp.325. Hbk \$39.95; pbk \$14.95.

IT WAS Winston Churchill who is reputed to have said: "The English never draw a line without blurring it". This comment would be a fair summary of David Vogel's verdict on the regulation of environmental policy in Britain: guidelines rather than rigid standards, persuasion through advisory committees rather than compulsion through the courts, "best practicable means" for the abatement of pollution rather than "best available technology". Behind this policy there is an important assumption, namely that the concept of zero risk is a mirage but acceptable risk is a reality. Politicians rely on this reality when they make regulations to control the exploitation of land, air and water. The result, to a non-British observer, is piecemeal policy which its supporters call pragmatic and its critics call sloppy. And, as David Vogel concludes after a scholarly examination of the evidence, it works quite well.

In the United States the policy for regulating the state of the environment is set out in sharp lines, no blurring. "The 1972 amendments to the Clean Water Act required all [*sic*] of the nation's waters to become 'fishable and swimmable by July 1, 1983'." Section 112 of the Clean Air Act amendments of 1970 required the Federal Environmental Protection Agency to regulate particularly hazardous pollutants to zero risk, with an "ample margin of safety" and regardless of cost. This policy, to a non-American observer, seems unrealistic, impracticable, and to offer a picnic to lawyers. But this policy, too, David Vogel concludes, works quite well. By "works quite well", I mean that there are better safeguards for health and amenity in Britain and the United States than there are in many other industrial societies. Risks are abated to what are, on the whole, levels acceptable to the public (though, when one considers the horrible harvest of road accidents in these countries, one is alarmed at the public perception of what constitutes "acceptable").

Vogel considers in detail the politics of control of pollution and land-use-planning in Britain and the United States. But his book is much more than a prosy "compare-and-contrast" essay. He uses his material to examine a much deeper issue, namely how governments in democratic countries can control the Externalities — the economist's technical word — produced by autonomous industry. And this

again is part of an even deeper issue: how to control the social behaviour of corporations. Both in Britain and the United States there has been progress in this policing of behaviour, but there are extraordinary contrasts in style. What is the reason for these contrasts? Why are there so few signs of convergence in means to achieve what are essentially the same ends?

Part of the answer to these questions is to be found in history. Britain suffered from the social misbehaviour of industry long before it affected the United States. By the year 1900 no fewer than 21 bills had been put before parliament for the abatement of smoke. Most of them failed because it could not be proved that smoke was actually a hazard to health, and in any case smoke-abatement would have been difficult and costly. But Britain was learn-

ing how to control the social behaviour of industry: in 1863, when the Alkali Act was passed to control the emission of hydrochloric acid from alkali works, it had the support not only of conservationists and landowners, but of the alkali industry itself. This was because the hazards of hydrochloric acid were evident and control was easy and cheap. The Act was administered by consensus, rarely by compulsion, although the Alkali Inspector had punitive powers. It would be an oversimplification to say that this set the pattern for regulation in Britain; what is more likely is that this pattern reflected the style of British politics over some — though not all — social issues, a style that seeks to persuade, to generate a willing compliance from the very people who are prone to unsocial behaviour. It is often criticized as a cosy consensus between the gamekeeper, paid to protect the environment, and the poacher, bent on exploiting it for his own profit. There is some justice in this criticism, but in the long run — perhaps because Britain is a small country where the leaders of industry belong to the same class as the leaders in politics — many industries do behave in a socially responsible way, induced to do so by desire to keep a bright public image fortified by fear of punishments held in reserve for intractable anti-social behaviour. What the critics call collusion between government and industry over industry's social behaviour is in fact sometimes genuine co-operation; the issues are settled over lunch in the club and subsequently validated by the issue of some regulation or guideline.

This is in striking contrast to the adversarial style of control in the United States where the impression is one of antagonism between the legislator and the industries against which he is legislating. The wrangles end up in the courts of law. At any one time there are hundreds of prosecutions going on. And no wonder. If the line is blurred its exact position can be a matter for compromise between non-lawyers. If the line is sharp and, as for the 1972 amendments to the Federal Clean Water Act, utterly unrealistic (it was estimated that it would have cost the entire gross national product of the United States for a year to achieve its declared intention), then of course there is hostility from industry and recourse to the courts. Vogel discusses one of the many difficulties that arise from this: that policy-making on environmental problems, which in Britain is still greatly influenced by scientific and technological experts, is, in the United States, in the hands of lawyers, who are less familiar with the scientific uncertainties and unsympathetic towards ambiguity. Hence some of the decisions of the courts in the United States are not the kind that would be made if

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Pollution past — industrial smokestacks in Pittsburgh in 1906. The picture is taken from Introduction to Environmental Science, 2nd Edn, by Joseph M. Moran et al., recently published by W.H. Freeman. Price is \$29.95, £29.95.

experts in environmental sciences had been in control.

Vogel does not pronounce judgement on the relative merits of the two systems of control. The American system is more time-consuming and expensive in legal costs. The British system relies on flexibility which can become mere laxity. Perhaps the secret of a successful policy is to be inflexible over principles (for example over the environmental quality standard ultimately desired) but flexible in the manner and pace at which one proceeds toward the desirable goal. Doubtless, on the statute book, the United States has the best-protected environment in the world; but the words of the statute book have not reached many of the rivers nor much of the atmosphere. The British statute book is more modest in its aspirations for the environment; but it does not often prescribe what could not be enforced without constant applications to the courts. Transferred to Vogel's larger canvas, this shows an interesting and (I suspect) controversial picture. Are the

mild British regulations, the preference for self-disciplined industry, signs of weakness in Westminster, or of a strong and respected government which can afford to rely on voluntary compliance? And is the American "reliance on rule-oriented enforcement" a sign that Washington can bring to heel unsocial behaviour in industry, or is it a sign of weakness in a government which does not command the loyalty of its industrial corporations?

It is no mere academic exercise to reflect on these questions. Expand Vogel's theme from environmental control to the control of trade-unions, corporation-cartels and local government, and you see its significance. Upon the answers to these questions depend the patterns of government Britain and the United States will have in the twenty-first century. □

Lord Ashby is a Fellow of Clare College, Cambridge CB2 1TL, UK. He was Chairman of the Royal Commission on Environmental Pollution from 1970 to 1973, and sometime member of the House of Lords Select Committee which deals with environmental directives from the European Economic Community.

Fuss over nothing

W.D. Hackmann

Leviathan and the Air-Pump: Hobbes, Boyle, and the Experimental Life. By Steven Shapin and Simon Schaffer. Princeton University Press: 1986. Pp. 440. \$60, £43.

The air-pump, more than any other scientific instrument, was the emblem of the revolution in physics that took place in the mid-seventeenth century. Other devices, such as the telescope, microscope, thermometer and barometer, preceded it, and all of them had a dramatic impact on how scientists conducted research and viewed the world. But the air-pump was the first mechanical device which made it possible for experiments to be carried out in a controlled laboratory environment — in this case, inside a glass bell-jar. It was also the seventeenth-century equivalent of "Big Science": very expensive, difficult to manufacture and temperamental to operate. Only a handful were made and each one was a prototype. The pump did become commercially available in the late 1670s, but it remained a costly instrument affordable only by wealthy virtuosi.

Until the middle of the seventeenth century, vacuum experiments were done in the small space above the mercury in the Torricellian barometer tube. Then, in about 1654, Otto von Guericke captured the public imagination with a startling performance of his "Magdeburg hemispheres"; two teams of burly horses could not pull the hemispheres apart after the air within them had been exhausted by the

first mechanical air-pump ever constructed. Robert Boyle's interest was aroused after reading about von Guericke's device in a book published in 1659, by the Jesuit Kaspar Schott, a prolific writer on scientific subjects. Boyle, and his assistant Robert Hooke, immediately set to work to develop a better pump. More significantly, Boyle began an experimental programme to delineate the properties of the vacuum inside the bell-jar of his pump. The results and conclusions were published in *New Experiments Physico-Mechanical Touching the Spring of Air* (1660), where Boyle consolidated his ideas about what he considered to be the proper experimental method (upon which much of our present-day science is based). *New Experiments...* touched off a series of controversies in the 1660s and 1670s that went far beyond what we would conventionally consider to be the realms of science. It is these controversies that are the subject of Shapin and Schaffer's impressive book.

The chief protagonists were Boyle and the philosopher Thomas Hobbes, one-time mathematics tutor to Prince Charles (Charles II). While Hobbes became best known for his political philosophy, expressed in *Leviathan* (1651), he also took a deep interest in science. The fullest statement of his philosophy of science is to be found in *Dialogus physicus* (1661), written in response to Boyle's experiments on the spring of air and here appearing for the first time in English translation.

Boyle distinguished between "obvious experiments" — simple observations of naturally occurring phenomena — and "unobvious" or "elaborate experiments" in which artificial phenomena were

produced by apparatus, in this case the air-pump. The difficulties faced by Boyle are not unfamiliar to the modern experimental scientist: problems with the physical integrity of his pump (leakage, for example), with standardizing or calibrating the phenomena, and with replication. All these matters led to disputes with his fellow scientists but, as Shapin and Schaffer demonstrate, the confrontation with Hobbes was much more profound and went to the heart of the experimental method. For Hobbes not only rejected the



Public display — a demonstration of Otto von Guericke's air-pump in Germany, in the mid seventeenth century, reproduced from *Technica curiosa sive mirabilia artis* (1687).

existence of the vacuum in nature but (to borrow from Wittgenstein) he played a different "language-game", arguing that only reason or philosophy, not Boyle's "engine philosophy", could yield reliable knowledge.

According to Shapin and Schaffer, this controversy was not so much about science as about social order. They examine the social conventions established for producing knowledge, and Boyle's techniques for making his experimental results acceptable to the scientific community. Thus (as the authors put it), the book is an exercise in the "sociology of scientific knowledge" and is written primarily for the community of scientific historians. Scientists may find this community's particular language-game at times rather off-putting, with its terms such as "literary technology" and "boundary-speech", and they will not find out how Boyle discovered the gas law (for which his air-pump was not necessary). But they should gain some thought-provoking insights into the complex relationship between experiment, theory, and the political and intellectual community. □

W.D. Hackmann is Assistant Curator at the Museum of the History of Science, University of Oxford, Broad Street, Oxford OX1 3AZ, UK.

Brain failure after eighty years

T.J. Crow

Normal Aging, Alzheimer's Disease and Senile Dementia: Aspects on Etiology, Pathogenesis, Diagnosis and Treatment. Edited by C.G. Gottries. *Editions de l'Université de Bruxelles, Avenue Paul Heger 26, Bruxelles, Belgium: 1985. Pp.308. FB 992.*

IN 1907, Alois Alzheimer described the thickening and tortuosity of fibrils within the neuronal cytoplasm of almost every fourth cell of the cerebral cortex of a 51-year-old woman who had been suffering from dementia. At that time he can have had little conception of the growth of interest in the phenomenon 80 years later. What may then have seemed an isolated pathological curiosity now appears as but one manifestation of an ubiquitous process whose relevance to ageing itself is the centre of discussion. What has become known as "Alzheimer-type dementia" is now generally agreed to include not only the unusual cases of presenile dementia (onset before 65 years of age) such as Alzheimer described, but also the great mass of dementias of old age. The prevalence of this condition is 5–8 per cent of the population at the age of 65, rising to 20 per cent at age 80 and 40–50 per cent above the age of 90 years.

The neuropharmacological aspects of the subject have been one of the features of recent research, and these proceedings record the contributions to two symposia at the Collegium Internationale Neuro-Psychopharmacologicum in Florence in 1984. Since 1976 it has been recognized that the cholinergic projections to the cerebral cortex are lost, giving rise to hopes that cholinergic replacement therapy might ameliorate some part of the psychological deficit. Such hopes are as yet unfulfilled. One problem, well documented in this volume, is that the cholinergic losses are far from isolated—there is evidence of loss of adrenergic and serotonergic projections as well. Whether the primary change occurs in the cell bodies of origin of the ascending systems, or whether these systems are affected secondarily to changes in the cortex itself (the temporal areas being a probable site of early change), is still a matter of debate, although opinion is swinging towards the latter possibility. Whichever viewpoint is correct, the anatomical and chemical selectivity of the process is surprising; in the cortex, for example, somatostatin-containing neurones are affected and cholecystokin and vasoactive intestinal polypeptide-containing cells are spared (see *Nature* **314**, 92–94; 1985). This may

yet provide a clue to the origin of the Alzheimer process.

Contributions to the book are not confined to neuropharmacology. They touch also on aetiology, both with respect to the enigmatic role of aluminium and to genetic factors (Alzheimer's disease shares with two other neurodegenerative conditions, Parkinson's disease and amyotrophic lateral sclerosis, a tendency to an earlier age of onset when there is greater incidence of familial cases). Aluminium is now known to be present in high concentration in the amyloid plaque, the structure which, together with the neurofibrillary tangle described by Alzheimer is the hallmark of the disease. Whether the element accumulates as a secondary effect or plays a pathogenic role remains unclear. In a short chapter, D.R.C. McLachlan speculates that aluminium could interact with calcium to contribute to the pathology. That electrolyte changes may be relevant to the aetiology of neurodegenerative disease is also suggested by Gajdusek and Garruto; they attribute the high incidence of amyotrophic lateral sclerosis and Parkinson's disease on Guam, the Kii peninsula of Japan and an area of New Guinea to low concentrations of calcium and magnesium in the soil, and the subsequent decline in incidence to correction of these mineral deficiencies. Such secular changes challenge the therapeutic nihilism which may be generated by the suspicion that these diseases, along with Alzheimer's dementia, are inseparable from the molecular genetic mechanisms of ageing.

This volume thus deals with a number of aspects of recent progress on Alzheimer's disease. There is relatively little reflection of the current interest in the protein structure of the plaques and tangles, but drug treatment strategies, although as yet of little practical value, are well covered. □

T.J. Crow is in the Division of Psychiatry, Clinical Research Centre, Northwick Park Hospital, Watford Road, Harrow, Middlesex HA1 3UJ, UK.

New editions

- *Microbes and Man, 2nd Edn*, by John Postgate (Penguin; pbk £4.95, \$6.95).
- *Methods in Plant Ecology, 2nd Edn*, edited by P.D. Moore and S.B. Chapman (Blackwell Scientific; hbk £34.80, \$57, pbk £23.80, \$36).
- *Statistics and Data Analysis in Geology, 2nd Edn*, by John C. Davies (Wiley; hbk \$52.55, £40.35, pbk \$24.95, £18.95).
- *Superfluidity and Superconductivity, 2nd Edn*, by D.R. Tilley and J. Tilley (Adam Hilger; hbk £36, \$60, pbk £18, \$25).
- *Partition of Cell Particles and Macromolecules, 3rd Edn*, by Per-Åke Albertsson (Wiley; \$59.95, £61.85).
- *Chromatographic Methods, 4th Edn*, by A. Braithwaite and F.J. Smith (Chapman & Hall; hbk £29, \$65, pbk £12.95, \$27).
- *The Colour Science of Dyes and Pigments, 2nd Edn*, by K. McLaren (Adam Hilger; £19.50, \$35).

Plenum:

Making It Happen

Forthcoming...

CHEMISTRY BY COMPUTER

An Overview of the Applications of Computers in Chemistry

by **Stephen Wilson**

In this wide-ranging survey, Dr. Wilson covers the techniques of computational chemistry and shows how they may be applied to problems ranging from interstellar chemistry to molecular biology.

0-306-42152-6/approx. 200 pp./ill./1986

DIGITAL RADIOGRAPHY

Selected Topics

edited by **James G. Kereiakes, Stephen R. Thomas, and Colin G. Orton**

Many of the significant developments resulting from the applications of physics and engineering to digital radiography are described here by noted authorities in the field.

0-306-42188-7/approx. 200 pp./ill./1986
\$39.50 (\$47.40 outside US & Canada)

PRINCIPLES OF ENDOCRINE PHARMACOLOGY

by **John A. Thomas and Edward J. Keenan**

Thomas and Keenan discuss a wide range of drug induced alterations in glandular secretions, drug-hormone interactions, and effects of drugs on clinical chemistry tests. Advances in recombinant DNA technologies are covered as they pertain to endocrine pharmacology, especially regarding recombinant human insulin and recombinant human growth hormone.

0-306-42129-1/hardcover/310 pp./ill./1986
\$39.50 (\$47.40 outside US & Canada)

0-306-42143-7/paperback/310 pp./ill./1986
\$22.50 (\$27.50 outside US & Canada)

PHARMACEUTICAL RESEARCH

Official Journal of the American Association of Pharmaceutical Scientists

Editor: **Wolfgang Sadee**

Pharmaceutical Research provides excellent research in all areas of pharmaceutical science including

- drug delivery
- computer-aided drug design
- biotechnology in drug development
- novel methods of drug analysis

Subscription: Volume 3, 1986 (6 issues)
Institutional rate:

\$89.50 in US/\$102.00 elsewhere

Personal rate:

\$45.00 in US/\$53.00 elsewhere

Plenum Publishing Corporation

233 Spring Street
New York, N.Y. 10013
In the United Kingdom:
88/90 Middlesex Street
London E1 7EZ, England

Reader Service No.13

Copies of articles from this publication are now available from the UMI Article Clearinghouse.

Yes! I would like to know more about UMI Article Clearinghouse. I am interested in electronic ordering through the following system(s):

- ☐ DIALOG/Dialorder ☐ ITT Dialcom
☐ OnType ☐ OCLC ILL Subsystem

☐ Other (please specify) _____

☐ I am interested in sending my order by mail.

☐ Please send me your current catalog and user instructions for the system(s) I checked above.

Name _____

Title _____

Institution/Company _____

Department _____

Address _____

City _____ State _____ Zip _____

Phone (____) _____

UMI Article Clearinghouse

Mail to: University Microfilms International
 300 North Zeeb Road, Box 91 Ann Arbor, MI 48106

NUCLEAR POWER AND ITS ENVIRONMENTAL EFFECTS

For the Layman, Nuclear Technician, Manager

Places the effects of possible nuclear radiation in perspective. Meets the need for a better understanding of radiation and its consequences especially after the TMI accident.

All vital aspects of nuclear power generation are considered, from fundamental principles to plant licensing, from uranium mining to disposal of waste. The safe operation of nuclear power plants, to prevent the escape of dangerous amounts of materials in case of an accident, is fully covered.

By Samuel Glasstone
 Walter H. Jordan
 American Nuclear Society
 555 No. Kensington Ave.
 La Grange Park, IL 60525
 USA

408 pages
 ORDER TODAY
 \$18.95 Softbound
 \$26.95 Hardbound

The source for the latest knowledge in Nuclear

On common ground in Scandinavia

Chris Stillman

The Caledonide Orogen — Scandinavia and Related Areas. Edited by D.G. Gee and B.A. Sturt. Wiley:1986. Two volumes, pp.1,266. £150, \$214.50.

LIKE most scientists, geologists work within a paradigm, and their main paradigm for the past quarter-century has unquestionably been the theory of plate tectonics. One of the fundamental concepts of this theory involves the formation of orogens — mountain belts — by the spreading and closing of ocean basins. The Caledonide Orogen evolved through the Palaeozoic period by the movements of North American, Baltic and European continents around a proto-Atlantic ocean; a full understanding of its development thus requires correlation of the rock units and their history throughout these extensive and now widely separated regions.

The decade 1974–1984 saw the flowering of one of the International Geological Correlation Programme's most successful ventures. This was Project 27, "The Caledonide Orogen", the objective of which was to establish a geological frame of reference for the whole orogen by encouraging researchers to get together and pool their knowledge. A series of annual meetings was arranged in each of the participating countries in turn, at which all concerned could meet, compare notes, deliver papers and visit the actual geological locations. In August 1981, Uppsala was the venue for the meeting at which the Scandinavian Caledonides were displayed, and the papers read there form the basis for this beautifully presented two-volume work.

This is a fine example of the book-maker's art — a lavish production, with excellent illustrations and extremely useful maps (the best being a 1:2,000,000 tectono-stratigraphic map of the Scandinavian Caledonides) — but its main value lies in the fact that it is the first major compilation of modern work on the Scandinavian Caledonides to become widely accessible. Rocks belonging to this orogen dominate Norway and much of Sweden; traditionally, the view taken on their geological history was determined by the perspective from either side of the national boundary (a point effectively made by the cartoon from 1896 illustrating the nappe controversy, here used to preface the section on regional geology). Until recently, publication in national and local journals tended to perpetuate the differences, hence the need for a comprehensive, integrated review. It is our good fortune that the review arose from an international

meeting, the proceedings of which could be published in clear English and made widely available to geologists throughout the world. We are doubly fortunate in that the editors represent both traditions; they have succeeded in pulling together a stylistically uniform and coherent review, and have produced a major step forward in Scandinavian geology.

Nevertheless, for all that the two volumes are a thorough and accurate reflection of how things stood in 1981, time has moved on. The whole style of approach to the problem has begun to change. Even before the publication of these books, awareness was growing of the logical improbability of achieving the orogen-wide correlation that was the *raison d'être* of the Project. Many now favour a way forward by applying terrane analysis, a strong hint of which is given in a short contribution by Duncan Keppie entitled "The Appalachian Collage". This, perhaps, increases the value of the books — in a subject which changes its ground rules so frequently, it is often difficult to achieve a correct perspective for judging the publications of previous years, but through the consistency of direction applied by the editors this compilation has a built-in spirit level.

The volumes are properly focused on Scandinavia, with 62 contributions dealing with the geology of the Scandinavian Caledonides, but the regional context is not forgotten and 22 other papers add wider-ranging though not particularly representative reviews. Indeed, while Greenland and Britain are obviously related areas, the addition of Sardinia, Kazakhstan and Mongolia is bizarre. The main body of the Scandinavian contributions is arranged with regional reviews followed by sets of papers on stratigraphy, structure, igneous rocks and metamorphism, each with introductory chapters. A final section includes various interpretations of the general tectonic evolution of the mountain belt. The reader wishing to obtain an overview need do little more than read the regional reviews and the introductory contributions to the subsequent sections. For the specialist, there is ample material in many of the individual papers, which are not just bare outlines of conclusions but properly present the supporting data. Access to the contents is greatly facilitated by the excellent subject index.

Those wondering whether or not to purchase this work should bear in mind that although the cost is great, so are the rewards. The coverage is high and wide, and the books themselves are handsome. Geologists around the world can learn much from them, and they may yet provide the seed for many more ideas on Caledonide orogenesis. □

Chris Stillman is an Associate Professor in the Department of Geology, Trinity College, Dublin 2, Ireland.

Ecological consequences of nuclear war

from S.J. McNaughton, R.W. Ruess and M.B. Coughenour

Simulations of the ecological effects on grassland ecosystems projected from the proposed climatic changes that would follow a nuclear war indicate that temperature and light reductions below ambient mean levels exceeding 10°C and 28%, respectively, are required for severe ecosystem deterioration to occur and that thresholds between promotion and decline are very abrupt.

ESTIMATES of the mass of smoke particles that would be injected into the atmosphere by fires following a nuclear war indicate that the resultant attenuation of light by suspended elemental carbon could substantially increase the optical depth of the atmosphere, producing major, rapid reductions of temperature and incident radiation at the Earth's surface¹, a phenomenon termed nuclear winter². Climatic models using various degrees of structural and dynamic detail have provided diverse predictions of the global climatic effects of atmospheric soot loading, but all are in general agreement that fires of the extent and intensity now reasonably projected to follow a nuclear war that included significant targeting of urban centres would lead to major falls in surface light intensities, temperature and, less certainly, precipitation³⁻⁵. More complex models have tended to predict both a longer duration and a broader geographical scale of climatic effects⁶. Transitory surface temperature reductions of more than 40°C and extended reductions of 20°C below mean ambient levels and light reductions to 0.01 and 0.1 of normal have been predicted. To place these estimates in perspective, the seventeenth century 'little ice age' is believed to have been caused by only a 1°C decline below mean ambient temperature⁷, and major glacial and interglacial periods may involve only 10–15°C peak-to-trough changes⁸. The potential biological and ecological consequences of the climatic changes projected to follow nuclear war could obviously be devastating^{9,10}.

Initial evaluations of the potential ecological effects of nuclear winter have been, of necessity, largely qualitative^{9,10}. Here we present quantitative results obtained by applying a wide range of the climatic effects currently predicted to result from atmospheric soot loading after a nuclear war to a documented, detailed, mechanistic simulation model of grassland ecosystem processes¹¹⁻¹³.

Procedures

We used nuclear winter climatic predictions to modify climatic sub-models of the

Grassland Research and Serengeti Systems Model¹¹⁻¹³, hereafter referred to as GRASS. It combines plant physiological processes; plant growth processes; plant morphometry; shoot demography; and grazing, with carbon, nitrogen, radiation and water sub-models. The principal focus of our research is the grazing ecosystem in the Serengeti region of Tanzania and Kenya¹⁵⁻¹⁸, so both biotic and abiotic parameters were drawn from studies of that ecosystem.

Experimental studies leading to parameter values have previously been documented¹⁹⁻²¹. Because the productivity of cultivation agriculture would probably be severely reduced after a nuclear war, forage agriculture and particularly non-subsidized rangelands, might be major food production systems for human survivors in non-combatant countries, many of which might be located in tropical-to-subtropical locations¹⁹.

We parameterized the GRASS simulations described here for mid-height grasses with a terminal canopy height of 50–150 cm, growing in an equatorial location at an elevation of 1,500 m with a mean annual rainfall of 793 mm and a rainfall-determined growing season of 296 days. We produced rainfall events probabilistically by GRASS and included all microclimatic parameters that might reasonably be modified by decreased light, lower temperatures and modified rainfalls. We ran diurnal variables at 2-h time steps and all others at 2-day time steps. For grazing simulations, we harvested the canopy uniformly to a height of 4 cm above the soil surface every 50 days, the optimum defoliation level for maximum mass yield to grazers in previous simulations¹².

The climatic predictions that have resulted from modelling nuclear war scenarios can be applied to ecological modelling in various ways. We chose the imposition of fixed changes on normal seasonal progression for the entire courses of growing seasons as we wanted to saturate a reasonable climatic factor space with ecological simulations that would produce response surfaces over as broad a range of conditions as was relevant, feasible and interesting.

We ran 90 full growing-season simulations, 45 each under grazed and ungrazed conditions, and modified precipitation by applying fixed percentages of –50, –30, –10, +10 and +30 to each rainfall generated by the weather simulator sub-model of the model. We simulated both increased and decreased rainfall because of the uncertainty of precipitation predictions from climatic modelling⁶. Because light intensity and temperature co-vary upon the injection of smoke into the atmosphere¹⁻⁶, we coupled values for those variables down to near freezing night-time temperatures, which terminated growth. Tropical pasture species are both chilling-sensitive and frost-intolerant, although species with ranges that extend outside the equatorial belt often have cold-tolerant populations in cooler habitats²²⁻²⁴. We did not simulate mean temperature reductions lower than 13°C once initial screening indicated that these temperatures produce periodic night-time frosts.

In addition to control conditions, we performed temperature- and light-intensity reductions at °C/% light combinations of –1°C/–6%; –2°C/–12%; –3°C/–16%; –5°C/–22% and –10°C at light reductions of –28%, –50%, –65% and –88%. The last two light reductions simulated atmospheric optical depths near 1 and 2, which might be widely distributed during post-war periods in non-combatant geographical locations¹⁻⁶. Although we did not apply a time-varying post-war trajectory to climatic variables, the typical seasonal patterns of GRASS were preserved, producing characteristic seasonal progression of precipitation, humidity, cloud cover and other normal weather variables. Control mean temperature was 21°C with a mean diurnal range from 28°C daytime highs to 14°C night-time lows. A given temperature reduction reduced maximum, minimum and intervening 2-h interval temperatures by that fixed amount. We superimposed radiation reductions on diurnal patterns and the cloud cover simulator and then fed them into sub-models of radiation and water balances to affect their performance and the resultant temperatures of plant tissues and

soils. Hence, we simulated total productivity, both above- and below-ground.

Baseline validation

Published data on below-ground primary productivity are scarce and our model produces reasonable outputs when compared with the available data¹¹. More widely available are data on above-ground productivity of ungrazed grasslands. We used three such data sets for a model validation internal to the simulations presented here. Data sets of above-ground primary productivities of ungrazed grasslands have been fit to regressions on annual rainfall for the Serengeti ecosystem¹⁸, eastern and southern Africa²⁵ and the world²⁶. There are many similar fitted curves in the literature but we used these three for baseline comparisons because they are both extensive, relevant to the ecosystem modelled, and have no data overlap.

The most complete data set for 52 worldwide sites²⁶ produces a best fit ($r^2 = 0.51$)-line of ANP = 0.5 (rain) - 29, where ANP is above-ground net primary productivity in $\text{g m}^{-2} \text{yr}^{-1}$ and rainfall is in mm. A Serengeti dataset for 20 locations subject to neither excessive drainage nor runoff water influx¹⁸ produces a best fit ($r^2 = 0.48$) line of ANP = 0.69(rain) - 102 and the other African dataset of 33 values²⁵ produces a line ($r^2 = 0.67$) of ANP = 0.85(rain) - 20.

Simulated above-ground productivities of ungrazed grasslands at ambient light and temperatures, and rainfalls less than 900 mm, above which both simulated and measured²⁷ productivities become asymptotic to the abscissa, produces a line ($r^2 = 0.97$) of ANP = 0.48(rain) - 48, as similar to the empirical lines as they are to each other. Together with previous comparisons of model performance with published empirical results¹¹⁻¹⁴, these results indicate that GRASS produces realistic grassland performance over a wide environmental range. Still, the application of post-war climatic predictions, themselves of uncertain surety, to any ecological model is a venture into uncharted predictive realms unamenable to experimental verification. But, where verification is feasible, GRASS performance is in good accord with empirical datasets and the climatic projections considered here deal mainly with conditions close to normal climatic variation, rather than extremes of extended cold, dark conditions.

Aftermath simulations

Our control conditions for simulations are 793 mm of rain at normal light and temperature; our baseline is the rainfall span at unreduced light and temperature. The total, above- and below-ground, simulated productivities of ungrazed grasslands are very sensitive to rainfall near

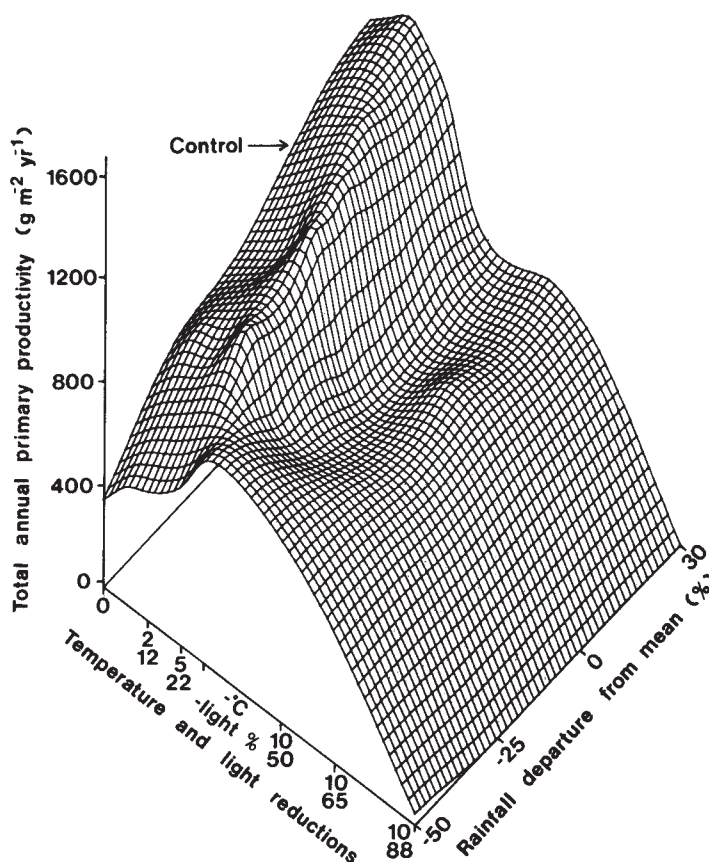


Fig. 1 Simulated total primary productivity in $\text{g m}^{-2} \text{yr}^{-1}$ of ungrazed grasslands in relation to deviations of precipitation and temperature-radiation environments from the long-term mean condition, the indicated by arrow labelled control. Note that the y-axis is linear in light intensity and non-linear in temperature. Data were fitted with the bivariate spline interpolation and graphed with the PROC 3D procedure of the 82.2 enhancements and update release of SAS/GRAPH (SAS Institute, Cary, North Carolina, 1982). Tilt, 60°; rotation, 50°.

normal light and temperature conditions, but are resistant to light and temperature departures from normal until mean reductions approach 28% and 10°C, respectively, below mean ambient levels (Fig. 1). This results in a substantial shelf where primary productivity is near or above control values for moderate climatic changes projected to follow a nuclear war. The upper level of the response surface is twisted by the tendency for cooler, darker conditions to compensate for reduced rainfall.

At the lowest rainfall level, in fact, primary productivity continues to increase at light-temperature reductions that significantly reduce production at higher rainfall levels. Therefore, reduced light and temperature have much greater water-conserving effects at low rainfalls than at higher rainfalls. These environmental interactions were expressed, in part, through compensatory changes in plant water potential, averaged over all shoots. Midway during the growing season, plant water potentials increase from -1.73 MPa at control light/temperature to -0.49

MPa at the 10°C/28% reduction under the lower rainfall conditions. At the highest rainfall conditions, the increase is only from -1.03 to -0.17 in this range.

Plant water potential regulates productivity by influencing net photosynthetic rates and tissue growth rates, and these compensatory interactions are propagated through GRASS performance. In addition, greater rainfalls increase leaf longevity, producing greater average ages of active canopy tissues, which are accompanied by declines in leaf nitrogen content and resultant rates of net photosynthesis. So no simple explanation suffices to explain the twisted response surface; rather, it is a complex consequence of plant growth and metabolic responses to interacting environmental variables. Because of the diagonal crest, productivity at high rainfall levels drops much more precipitously as the more severe region of the factor space is penetrated. The greatly reduced light levels indicate that the grasslands have a broad ability to compensate for light reductions after the initial drop, producing a shelf extending to the light

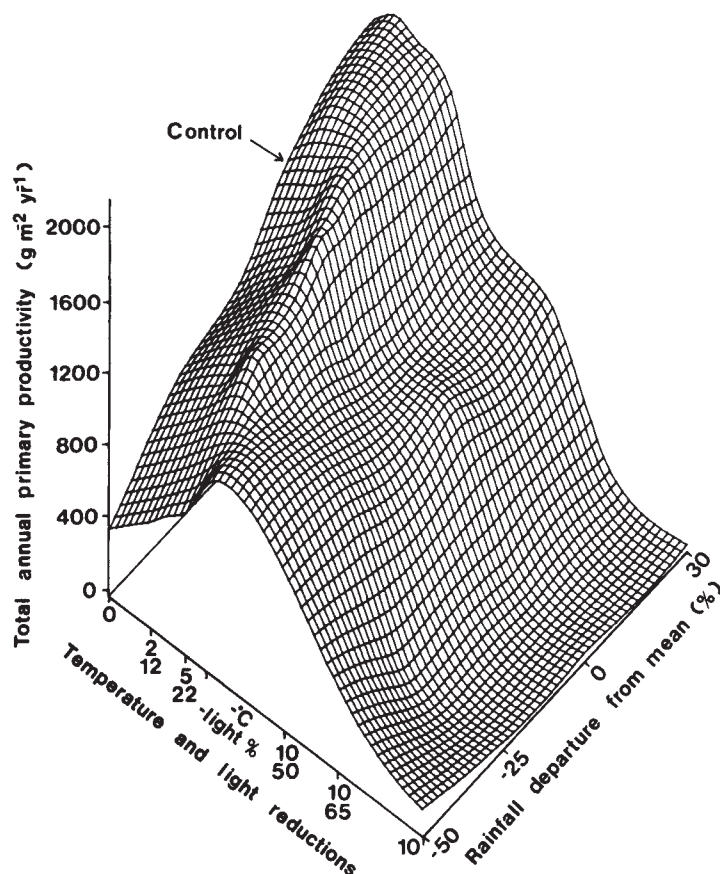


Fig. 2 Simulated total primary productivity in $\text{g m}^{-2} \text{yr}^{-1}$ of grazed grasslands in relation to deviations of precipitation and temperature-radiation environments from the long-term mean condition, indicated by an arrow. Note that the z-axis scale differs from Fig. 1 if comparing the two response surfaces. Data were fitted and plotted as described in Fig. 1 legend. Aspect as in Fig. 1.

intensities produced by an atmospheric optical depth of one. At that optical depth, productivities are 79% of baseline at low rainfall levels, 61% at normal levels, and 46% at the highest simulated rainfall. Beyond an optical depth of 1, however, productivity again plunges precipitously to near zero at an optical depth of 2 and this surface of steeply decreasing primary productivity was rainfall-independent.

More interesting than the obvious fact that severe temperature and light reductions will drive production to zero is the finding that productivities are increased by most simulated post-war light and temperature reductions of moderate extent at a broad range of rainfalls. The minimum productivity on the upper response shelf, equivalent to 41% of control, is produced by the 50% rainfall reduction with no changes in temperature and light. The maximum productivity of the ungrazed simulations, 44% above control, occur at the highest simulated rainfall level accompanied by temperature and light reductions below ambient of 2°C and 12%, re-

spectively. The diagonal crest is produced by the tendency for very minor light/temperature reductions to produce maximum productivities at higher rainfall levels, whereas low rainfall systems continued to exhibit increased productivity at substantial light/temperature reductions. Beyond this crest productivity in low rainfall simulations declined steadily to zero levels, lacking the shelf that occurs at higher rainfalls. These results have important implications for potential yields from water-limited ecosystems in post-war environments.

Grazing stimulates the primary productivities of grasslands at higher rainfall levels but not at low rainfalls; as a consequence, the overall grazed response surface has simpler but much steeper contours (Fig. 2). Primary productivity has a more convex shape under grazed than ungrazed conditions because of higher peaks, steeper contours and more abrupt thresholds. The diagonal crest on the upper shelf is more pronounced because of the tendency for primary production at high rainfall to decline after a slight rise

with minor light/temperature reductions and an extension of the ameliorating range of those reductions at the lowest simulated rainfalls. Net primary productivity is stimulated a maximum of 38% above control conditions by the combination of highest rainfall with a light/temperature reduction of just 1°C and 6%. At baseline conditions, the lowest simulated rainfall reduces the productivity of grazed grasslands to only 28% of the value at control conditions. There is little evidence of a shelf of resistance to light reductions at temperatures above freezing.

Grazed grasslands are generally able to maintain reasonable levels of primary productivities down to a light reduction of 50% below normal, but they are severely inhibited by the reductions produced by an atmospheric optical density of 1. At that level, primary productivities are 19% of baseline at low rainfalls, 12% at normal rainfalls, and 9% at the highest rainfall simulated. Compared with the ungrazed simulation, this result suggests that the trophic web would collapse under conditions still allowing significant primary productivities in ungrazed grasslands.

The ameliorating effects of light and temperature reductions produce steeper responses under grazed conditions over the upper productivity shelf. For example, at control rainfall the productivity of ungrazed systems is 17% below normal at the $10^{\circ}\text{C}/28\%$ temperature/light reduction. In the grazed ecosystem, in contrast, net primary productivity under those conditions is still 13% above baseline. As a consequence, the descent of productivity as the limiting factor space is penetrated is much more precipitous for grazed than for ungrazed ecosystems.

As grasses must be converted into animal products for uncultivated vegetation in grassland climates to support human populations, secondary productivity is of particular importance to consideration of the potential consequences to humans of climatic changes that might follow a nuclear war. There are two fundamentally different approaches to estimating secondary productivity from model output. The simplest, but perhaps least revealing, is to convert the plant mass consumed by grazers to animal biomass by assuming some conversion efficiency based on animal feeding trials²⁸. That approach assumes that forage quality does not vary across the range of conditions simulated and has the virtue of converting forage consumption per unit area directly into animal yield per unit area.

When we applied a 10% conversion efficiency^{28,29} to animal forage consumption output from GRASS, with that output varying according to both forage amount and its availability due to canopy geometry¹², we found ($r^2 = 0.87$, $P < 0.001$)

that secondary productivity = $0.052 \times$ total primary productivity, the units of productivity are in $\text{mass area}^{-1} \text{yr}^{-1}$ for the 40 environmental combinations above zero. Secondary productivity then, under this assumption, would reproduce the response surface of Fig. 2, but with absolute values 95% lower on a mass density basis.

A second and, we believe, more realistic approach to estimating secondary productivity is to account for differences in forage quality. Both wild³⁰ and domestic³¹ ungulates, particularly in tropical locations, are more often limited by forage quality than by its quantity. Higher mass productivities produced by GRASS are generally accompanied by lower forage quality because nutrients such as nitrogen are diluted by carbon accumulation in plant tissue, consistent with real ecosystems³². Over the range of non-zero conditions simulated here, the relationship between the nitrogen content of forage consumed and the mass of carbon that is consumed is

$$\%N = 1.93 - 0.00386 \text{ gC}$$

where $r^2 = 0.92$, $P < 0.001$ and gC is grams of carbon consumed per square metre of land surface per year.

As a consequence of this negative relationship between forage quality and mass availability, the amount of N consumed was asymptotically related to the amount of forage carbon consumed ($r^2 = 0.86$, $P < 0.001$) with $\text{gN} = (2.27 \ln \text{gC}) - 5.2$ where units are masses of N and C $\text{m}^{-2} \text{yr}^{-1}$. This conundrum relating forage quality and quantity of further exacerbated by a positive relationship between forage nitrogen content and the digestibility of that nitrogen (ref. 33). Therefore, as a more realistic approach to estimating potential secondary productivities of post-war ecosystems, we multiplied N yield to grazers in $\text{gN M}^{-2} \text{yr}^{-1}$ by the digestibility of that N predicted from the linear relationship between forage N content and digestibility of N (ref. 33). This yielded digestible $\text{gN m}^{-2} \text{yr}^{-1}$ consumed by grazers. Additional assumptions that we are unwilling to make at this stage would be required to convert the areal yield of digestible N to animal production, but the shape of the response surface is more likely to reflect the secondary productivity potentials of post-war ecosystems than the simple conversion from Fig. 2.

The quality-based secondary productivity response surface produced by GRASS varies markedly from both primary productivity surfaces (Fig. 3). Maximum secondary productivity, 38% above the control value, is produced at moderate drought and no reduction of ambient light and temperature. Minimum secondary productivity potential outside the severe factor space is at the highest rainfall with temperature-light reductions of $5^\circ\text{C}/22\%$, where the value is only 36% of the control. This response surface is caused by a

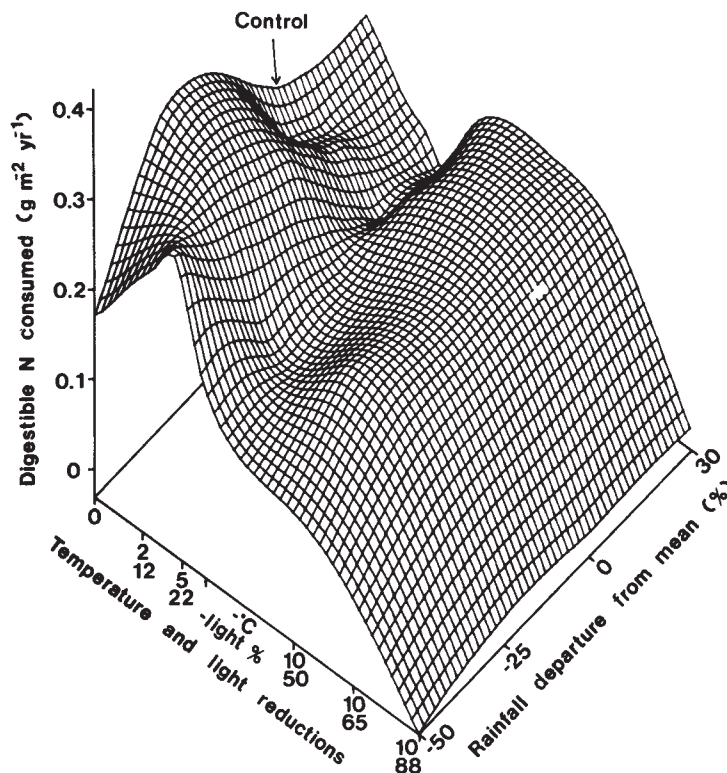


Fig. 3 Simulated yield of digestible N to grazers in $\text{g m}^{-2} \text{yr}^{-1}$ in relation to deviations of precipitation and temperature-radiation environments from the long-term mean condition, indicated by an arrow. Data were fitted and plotted as described in the legend for Fig. 1. Aspects as in Fig. 1.

greater reduction in plant-carbon assimilation than of nutrient assimilation by mild environmental stress, a phenomenon documented empirically^{34,35} and that GRASS reproduces. As a consequence, there was a pronounced peak of secondary productivity potential at a rainfall reduction to 30% below normal in baseline light and thermal environments. Values drop precipitously around this optimum, particularly at lower rainfall levels and no light/temperature reduction. But the surface is convoluted with folds and pockets, especially as severely limiting conditions are approached. Reduced light levels at non-freezing temperatures produce a broad secondary shelf of slightly increasing secondary productivity potential with a reversal of the rainfall field leading to higher potentials at higher rainfalls. At light intensities above half normal, potential secondary productivity drops sharply to zero. Compared with control conditions, all higher rainfall simulations and all reduced light and temperature conditions under the normal rainfall regime result in secondary productivity potential lower than the control. But many of the climatic conditions predicted to follow a nuclear war, that is, combinations of reduced rainfall with cooler, darker con-

ditions, result in significant enhancements in the yield of digestible N to herbivores.

When compared with the primary productivity simulations and simple extrapolations of secondary productivity from them, these simulations highlight two significant features of ecosystems. First, countervailing processes make it difficult, if not impossible, to optimize all performance standards simultaneously. Second, simple extrapolations are incapable of providing meaningful estimates of the effects of complex biological responses to environmental variation.

Implications

We make no claim for the ultimate accuracy with which GRASS simulations could predict the ecological aftermath of a nuclear war. But we do believe that those projections are fundamentally realistic; that they provide reasonable boundary conditions for severe declines in ecosystem carrying capacity; and that they prudently reflect the ecological conditions that could be expected to occur in many grassland and other water-limited ecosystems following such a war. Primary productivities under both grazed and ungrazed conditions, and secondary productivity potentials, are stimulated by cli-

matic conditions that would be relatively severe under normal conditions, although they can be characterized as mild compared with the more extreme nuclear winter conditions predicted by some models. The abrupt interfaces between stimulatory and degradative effects emphasize the hazards associated with deductions from imprecise climatic predictions.

The sensitivity of our simulations to the countervailing effects of precipitation and radiation-temperature changes within the non-severe factor space indicates that it would be profitable to devote more effort in climatic modelling to precise definitions of the coupling between precipitation and radiation-thermal regimes, perhaps at the sacrifice of global spatial scales where the models are in general, if not precise, agreement. Because climatic effects have post-war meaning only through their ecological effects, more attention to ecological modelling is clearly also warranted.

Our simulations indicate several areas where additional empirical research could contribute fruitfully to a better understanding of both the potential ecological consequences of nuclear war and basic ecological knowledge. We terminated temperature reductions at levels just above those producing periodic nighttime frosts as tropical pasture plants are both chilling sensitive and frost intolerant²²⁻²⁴. However, strains of a widely cultivated tropical grass, *Cynodon dactylon*, have been produced that are tolerant of winter conditions to near 40° N in central North America³⁶, and tropical species with ranges extending outside equatorial locations have frost tolerant genotypes^{22,23}. This suggests that there is sufficient genetic variability in tropical grasses to produce freeze-tolerant genotypes, and natural selection accompanying severe nuclear winter would be intense. If some tolerant genotypes survived as either dormant individuals or as seeds, ecosystem recovery might be possible following even the most severe nuclear winter. We know of no extensive evidence on the freeze tolerance of drought-dormant rather than

actively growing tropical grasses. Nuclear winter during the tropical dry season might not result in extensive plant mortality if drought-hardened individuals are also cold tolerant; information on the relationships between these adaptive traits would allow more realistic estimates of the expected recovery potentials of tropical and subtropical ecosystems if severe nuclear winter penetrated those geographical regions. Information is also limited on the seed banks of tropical soils and the freeze tolerance of those seeds²³. Nuclear winter would expose seeds to cold, dry, dark environments, which in many cases are optimal for increasing seed longevity.

Secondary productivities can also be influenced by many factors in addition to the limitations imposed by food quantity and quality. Radiant heat load³⁷, availability of water, wind speed²⁸, mineral nutrient availability^{31,33} and the shield of veterinary practices protecting both wild and domestic ungulates from potentially epidemic disease^{38,39} could all be modified by post-war conditions. In addition, cold rains accompanying periodic smoke cover and chilling that precipitates water out of the atmosphere might have devastating effects on tropical animals. Realistic estimates of such ecological effects would add precision to estimates of the post-war carrying capacity of ecosystems.

But even with the many uncertainties, we believe that the results of simulations with GRASS have three principal implications for the carrying capacities of ecosystems were a nuclear war to modify climate in the directions now envisaged. First, mild (by the standards of nuclear winter) climatic changes tending to reduce heat loads and evaporative demands of the atmosphere might increase the productivities of many water-limited ecosystems. Such ecosystems occupy major expanses of Earth's surface.

Second, distinctly different conditions lead to performance optima at different levels in the trophic web. Those optima, therefore, cannot be attained simultaneously. Grain production, for example,

would probably track the response surface that we obtained for primary productivities of ungrazed vegetation. Because grassland climates are centres of grain production, increases in precipitation and/or reduced evaporative demands could tend to increase substantially the grain production potential of post-war ecosystems. Animal production, on the other hand, would probably be inhibited by the same conditions.

Third, and, we believe most important, the transition between non-catastrophic (even favourable) environmental changes and those that are calamitous may be extremely abrupt. The presence of those thresholds in all three simulation results suggests that the ecological effects of a nuclear war are very unpredictable and may change drastically over narrow environmental ranges. A population of human survivors in tropical-to-subtropical locations might encounter an increased carrying capacity for certain climatic perturbations only to be plunged into destruction by a seemingly minor drift away from those conditions. Overall, our results emphasize the uncertainty that must be attached to any predictions about the ecology of a post-war Earth, and suggest that ecological unpredictability itself could be one of the most damaging consequences of post-war climatic effects.

Development of GRASS was funded by the US National Science Foundation's Ecosystem Studies Program. Its application here was funded by Syracuse University. That application was prompted by two workshops sponsored by the ENUWAR unit of SCOPE, University of Essex (attended by S.J. McN.) None of those organizations necessarily endorses the conclusion of the research reported. □

S.J. McNaughton and R.W. Ruess are at the Biological Research Laboratories, Syracuse University, 130 College Place Syracuse, New York 13210, USA; and B.M. Coughenour is at the Natural Resources Ecology Laboratory, Colorado State University, Fort Collins, Colorado 80523, USA.

- Crutzen, P.J. & Birks, J.W. *Ambio* **11**, 114–125 (1982).
- Turco, T.P., Toon, O.B., Ackerman, T.P., Pollack, J.B. & Sagan, C. *Science* **222**, 1283 (1983).
- Covey, C., Schneider, S.H. & Thompson, S.L. *Nature* **308**, 21–25 (1984).
- Crutzen, P., Galbally, I.E. & Bruhl, C. *Climatic Change* **6**, 323–364 (1984).
- Thompson, S.L. *et al.* *Ambio* **12**, 236–243 (1984).
- Pittock, A.B. *et al.* *Environmental Consequences of Nuclear War Vol. 1* (Wiley, Chichester, 1986).
- Parker, G. *Europe in Crisis 1598–1648* (Harvester, Sussex, 1980).
- Heusser, C.J., Streeter, S.S. & Stuiver, M. *Nature* **294**, 65–67 (1981).
- Ehrlich, P.R. *et al.* *Science* **222**, 1293–1300 (1983).
- Harwell, M.A. & Hutchinson, T.C. *Environmental Consequences of Nuclear War*, Vol. 2 (Wiley, Chichester, 1985).
- Coughenour, M.B., McNaughton, S.J. & Wallace, L.L. *Ecol. Modelling* **23**, 101–134 (1984).
- Coughenour, M.B., McNaughton, S.J. & Wallace, L.L. *Ecol. Modelling* **26**, 177–201 (1984).
- Coughenour, M.B. *Ecol. Modelling* **26**, 203–220 (1984).

- Coughenour, M.B. *Ann. Mo. Bot. Gard.* **72**, 852–963 (1985).
- McNaughton, S.J. *Science* **191**, 92–94 (1976).
- McNaughton, S.J. *Am. Nat.* **113**, 691–703 (1979).
- McNaughton, S.J. *Ecol. Monogr.* **53**, 291–320 (1983).
- McNaughton, S.J. *Ecol. Monogr.* **55**, 259–294 (1985).
- McNaughton, S.J., Wallace, L.L. & Coughenour, M.B. *Ecology* **64**, 307–318 (1983).
- Ruess, R.W., McNaughton, S.J. & Coughenour, M.B. *Oecologia* **59**, 253–261 (1983).
- Coughenour, M.B., McNaughton, S.J. & Wallace, L.L. *Afr. J. Ecol.* **23**, 179–194 (1985).
- Clements, R.J. & Ludlow, M.M. *J. appl. Ecol.* **14**, 551–566 (1977).
- Humphreys, L.R. *Environmental Adaption of Tropical Pasture Plants* (Macmillan, London, 1981).
- Larcher, W. & Baurer, H. in *Physiological Plant Ecology I, Encyc. Plant Phys.* Vol. 12A (eds Lange, O.L., Nobel, P.S., Osmond, C.B. & Ziegler, H.) 403–437 (Springer, New York, 1981).
- Deshmukh, I.K. *Afr. J. Ecol.* **22**, 181–186 (1984).
- Lauenroth, W.K. in *Perspectives in Grassland Ecology* (ed.

- French, N.) 3–24 (Springer, New York, 1979).
- Sims, P.L. & Singh, J.S. *J. Ecol.* **66**, 573–597 (1978).
- Garrett, W.N., Bond, T.E. & Kelly, C.F. *J. Anim. Sci.* **19**, 60–66 (1960).
- Lodge, G.A. & Lamming, G.E. *Growth and Development of Mammals* (Butterworths, London, 1968).
- Sinclair, A.R.E. *J. Anim. Ecol.* **44**, 497–520 (1975).
- McDowell, L.R., Conrad, J.H., Ellis, G.L. & Loosli, J.K. *Minerals for Grazing Ruminants in Tropical Regions* (Univ. Florida, Gainesville, 1983).
- Vitousek, P. *Am. Nat.* **119**, 553–572 (1982).
- McDonald, P., Edwards, R.A. & Greenhalgh, J.F.D. *Animal Nutrition* (Longman, London, 1973).
- White, T.C.R. *Oecologia* **22**, 119–134 (1976).
- Mattson, W.J. *A. Rev. Ecol. Syst.* **11**, 119–161 (1980).
- Beard, J.B. *Turfgrass: Science and Culture* (Prentice-Hall, Englewood Cliffs, New Jersey, 1973).
- Garrett, W.N., Givens, R.L., Bond, T.E. & Hull, J.L. *Proc. Western Sect. Am. Soc. Anim. Sci.* **17**, 349–355 (1966).
- Rossiter, R.B. *et al.* *Vet. Rec.* **113**, 459–461 (1983).
- Rossiter, P.B. *et al.* *Prev. Vet. Med.* **1**, 257–264 (1983).

Laboratory investigation of the electrodynamics of rock fracture

B. T. Brady & Glen A. Rowell

Denver Research Center, US Department of the Interior, Bureau of Mines, Denver, Colorado 80225, USA

Light produced by the failure of rocks subjected to uniaxial compression in various atmospheres has been analysed spectroscopically in the visible and near-infrared. In each case, the observed spectrum was unique to the ambient atmosphere. Spectrographical and electromagnetic investigations support an exoelectron bombardment mechanism to explain the observed light emission. Several implications of this result are discussed.

OBSERVATIONS of light emissions and other lower-frequency electrical phenomena associated with rock fracture in mines and earthquakes have been reported for several centuries¹⁻⁴. These reports did not attract serious scientific attention until photographs of light emissions were obtained during the 1965 Matsushiro, Japan, earthquake swarm¹. Although many theories as to the source(s) of these light emissions have been presented¹⁻⁶, it is only within the past few years that serious attempts have been made to conduct laboratory investigations of this phenomenon⁷⁻¹². Due to the absence of precise experimental data, the lack of consensus regarding the possible explanations of the light-producing mechanism(s) from fracturing rock is understandable. The mechanisms most commonly proposed⁵ include: (1) rock fragments frictionally heated to incandescence; (2) electrostatic discharge produced by the deformation of piezoelectric minerals or due to charge separation on fractured surfaces; (3) plasmas produced by rapid and intense heating of rock material; and (4) excitation of the ambient atmosphere by particle (electron and/or positive or negative ion) bombardment.

As each of the above mechanisms produces an optical spectrum having characteristic features, spectroscopic analysis in the visible region (10^{14} – 10^{15} Hz) offers an experimental means of elucidating the process by which the light is produced. For example, rock fragments frictionally heated to incandescence would produce a continuous spectrum approximating that of a black-body radiator at a temperature corresponding to that of the heated rock. An electrostatic discharge caused by deformation of piezoelectric minerals would normally produce line spectra characteristic of the excited elements: little or no background continuum would be observed, and transitions from both neutral and ionized states might occur. In addition, because this type of excitation can be best categorized as 'spark-like', microwave emanations would be expected. A plasma originating from the rock sample during catastrophic failure would produce a background continuum closely approximating that of a black-body radiator. Atomic emission lines, characteristic of electromagnetic interactions occurring within the plasma, would be superimposed on this spectrum. Transitions from neutral as well as ionized species might also be observed. The plasma temperature could be inferred from the ratio of the line-to-background intensities¹³. Finally, excitation of the ambient atmosphere by particle bombardment would produce molecular and atomic line spectra. The spectrum would be determined by the chemical properties of the atmosphere as well as by the energy, mass and charge of the particles. Spectroscopic examination and characterization of the emitted light should determine which, if any, of the above models are valid.

Experimental philosophy

Experimental conditions and materials were chosen to enable positive differentiation between the four proposed light causing mechanisms. Five ambient atmospheres were chosen: argon,

helium and air at atmospheric pressure; water and a vacuum of 1×10^{-6} torr.

The monatomic rare gases, argon and helium, were selected because of their relatively simple atomic line spectra¹³. These gases have been the subject of extensive spectroscopic investigations and the energy levels of both neutral and ionized species are well established. Because the first ionization potentials for argon and helium are 15.8 and 24.6 eV, respectively, the minimum energy available to the excitation process can be deduced from the observed transitions.

The high-vacuum ($\sim 10^{-6}$ torr) experiments were designed to determine whether the emitted light originated in the ambient atmosphere or in the rock material. Since the mean free path of nitrogen and oxygen is $\sim 10^4$ cm at 10^{-6} torr and 25 °C, vacuum conditions would seem to preclude the production of light from collisional excitations¹⁴. Should light be observed during rock fracture in the vacuum experiments, then either the ambient atmosphere (gas remaining in the vacuum chamber) is being excited by a mechanism other than an electric field or the rock material itself (including any excited gases that might exist within the rock pores) is the source of the light.

Water was chosen as an ambient fluid because a liquid has a much higher density than does a gas, an important consideration if collisional processes cause the light emission. Additionally, tap water is a relatively good conductor ($\sim 200 \mu\text{S}$)¹⁴ and would minimize the possibility of any capacitive charge buildup caused by piezoelectric minerals present on the sample surface.

A fine-grained granite from Salida, Colorado, and a fine-grained basalt from Table Mountain near Golden, Colorado, were the two rock materials chosen for the tests. Basalt was chosen specifically because of the absence of quartz or other strongly piezoelectric minerals. All rock samples except those used in the high-speed movies were drill cores, of diameter 2.2 cm and length 5 cm. All samples were prepared to ASTM standards.

Preliminary experimental results

Figure 1 illustrates the typical fracture behaviour of a 5.4-cm-diameter $\times$ 10.8-cm-long specimen of Salida Granite from high-speed movies (3,000 frames s^{-1}). Several observations are noteworthy. First, the dust cloud (composed of finely ground rock particles) forms during the main shock ($t = 0$) and not as a result of frictional sliding along crack (fault) surfaces. Second, the loading platens exhibit no significant motion until the sample has disintegrated; that is, the stiffness of the loading system may be irrelevant to the fracture process in brittle materials. Third, the process of fracture in brittle materials, such as rock, is explosive. Implications of these observations will be discussed elsewhere (in preparation).

Figure 2 illustrates typical light emission from rock fracture. This photograph was obtained using a night-vision scope. Generally, light produced by rock fracture can be viewed only in

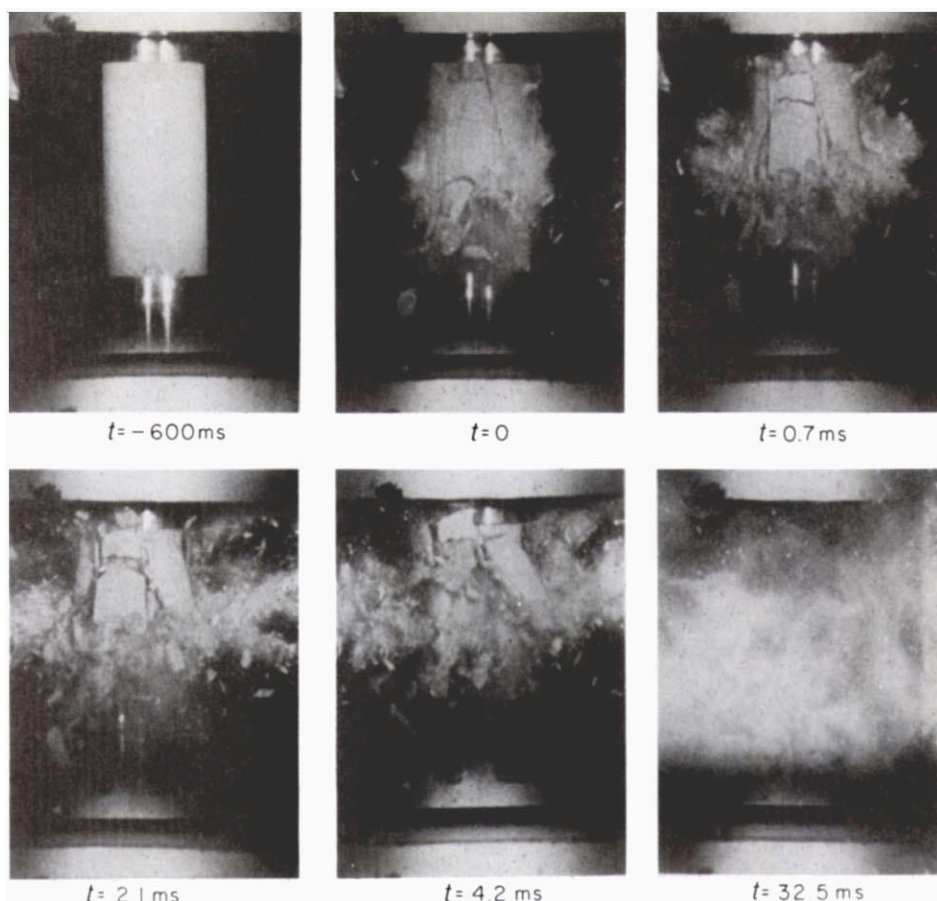


Fig. 1 Sequence of high-speed ($3,000 \text{ frames s}^{-1}$) photographs illustrating fracture of Salda Granite.

darkness by an observer with night-adapted vision. Photodiode experiments show that the light emission is composed of a few discrete pulses, each persisting for only $\sim 100 \mu\text{s}$. Figure 3 illustrates typical photodiode and applied load outputs versus time for the two rock types studied in this investigation. (Note that the response time of the PIN (positive/insulated/negative) diode and recording system is $\sim 1 \mu\text{s}$, and the fall time is much slower because of the amplifying electronics.) Therefore, the major problem of this investigation was low light intensity of short duration. This point is amply shown in Fig. 4a, b. Figure 4a shows a sample of Salda Granite photographed during catastrophic failure and illustrates the formation of the 'dust' plumes and other larger pieces of rock in the process of spalling. Of particular interest is the nature of the 'dust' plumes emanating along fracture surfaces. In several fractures, the plumes occur only along discrete segments, with no plumes occurring elsewhere along the fracture surface. Figure 4b, taken through a single-stage image intensifier, illustrates the light emission emanating from a different sample of Salda Granite and supports the observation that the light duration is short ($< 10^{-3} \text{ s}$); if the light duration were greater than several milliseconds, discrete emissions would be smeared. In addition, the discrete nature of the light emissions along selected fracture surfaces is evident and similar to the plume distribution shown in Fig. 4a.

Experimental procedures

The final design of our spectrographic system resulted from these preliminary tests and much trial and error. Because of the low light intensity and short duration, priority was given to optical speed, with resolution a secondary consideration. A variable slit prism spectroscope with a two-stage image intensifying system (two night-vision scopes mounted in series) was used to observe spectra. The image formed by the spectroscope was focused on the photocathode tube of the first stage intensifier.

The final image was then viewed directly on the screen of the second stage or recorded photographically.

Because light emission from rock failure has been the subject of considerable controversy, it was important to acquire photographic spectra. In addition to the short time duration and low intensity of the light produced by rock fractures, the problem

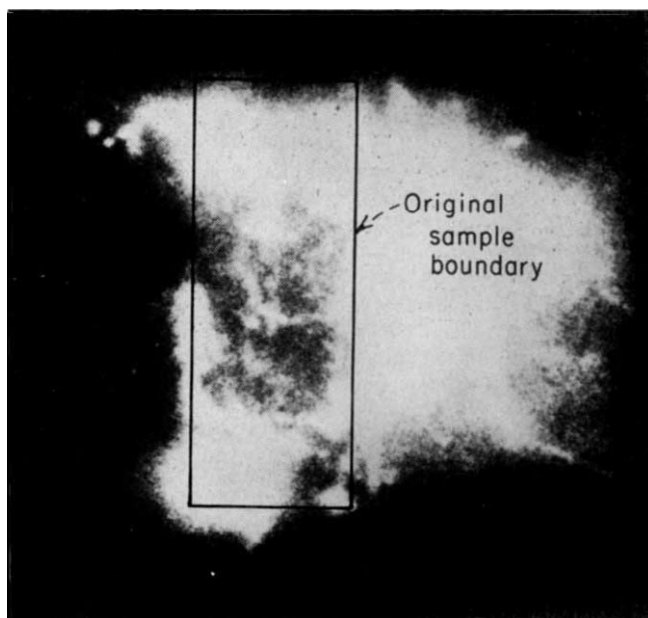


Fig. 2 Typical light emission observed from fracture of Salda Granite.

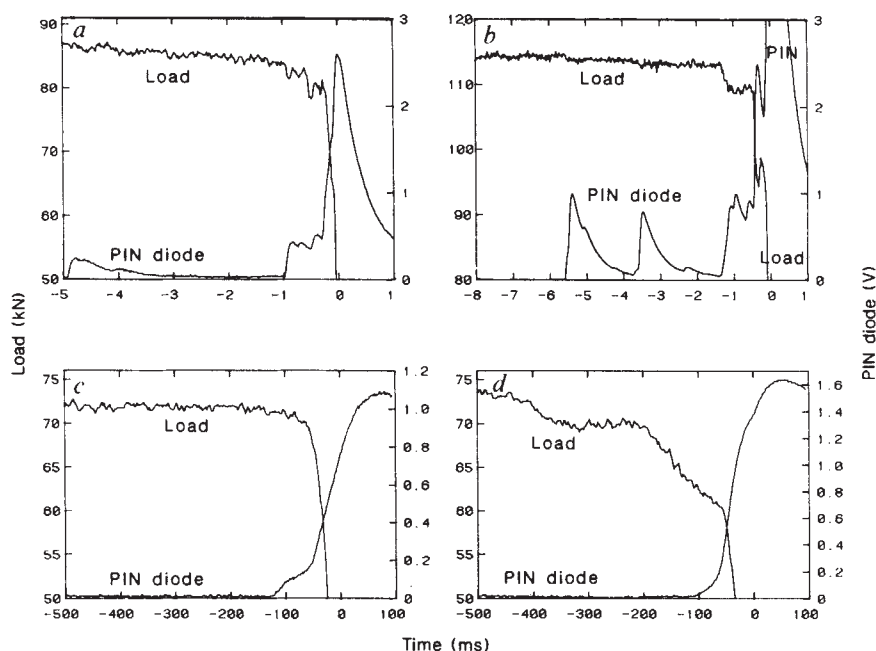


Fig. 3 Typical photodiode and applied load outputs versus time for Salida Granite (a, b) and Table Mountain Basalt (c, d).

of photographing a spectrum was complicated by inexact knowledge of when the rock sample would fail and the unfavourable signal-to-noise ratio of operating both image intensifiers at maximum gain. On the other hand, operating the system at less than maximum gain produced images too faint to photograph.

The spectra were finally recorded on Polaroid type 612 film, with an ASA rating of 20,000. The ultra-high speed of this high-contrast black and white film made it ideally suited to our experiment. Accordingly, an XL Graflex camera with a Polaroid back was placed at the exit of the second image intensifier to take photographs. With both image intensifiers set at maximum gain, 1/10 s at $f/5.6$ was the longest exposure able to produce acceptable images compared with the background noise. Output from a load cell was used to activate a digital oscilloscope which sent a pulse to a circuit which activated a low-mass solenoid which triggered the camera shutter. This 'Rube Goldberg'-type system afforded a probability of ~ 0.5 of the camera lens being open during rock failure to record the spectra of any light emission.

The rock specimens were isolated in a cylindrical (7.6 cm inner diameter) Lexan chamber to prevent damage to the spectroscope. To maintain a gas-tight seal, a sliding steel disk at each end of the chamber was fitted with O-rings. Brass platens were placed between the rock sample and the steel disks. The chamber assembly was then placed in the press and a slight force applied to align the components. Next, gas was introduced into the chamber through one port, and the end of a tube from the exit port was placed under water to maintain a slight positive pressure within the chamber. The laboratory was then darkened and the spectrum from a reference lamp recorded on film. The camera shutter and solenoid were reset, the oscilloscope trigger was armed, and the image intensifiers were turned to maximum gain. By this time, the chamber had been sufficiently flushed of air, and the sample was loaded rapidly (strain rate $\dot{\epsilon} \approx 10^{-2} \text{ s}^{-1}$) to failure. The photographic print was removed from the film pack and developed.

Results

Figure 5a is the spectrum resulting from the fracture of Salida Granite in argon. The reference spectrum at the top of the photograph was produced by a low-pressure argon lamp. Several prominent lines are identified. Of particular importance is the observation that no spectral lines are produced by the failure of Salida Granite in argon other than the lines present in the

reference spectrum. All lines observed are produced by neutral atomic argon. No transitions from ionized states are observed. The 7,635-Å line is prominent, and the fact that the upper state of this transition lies 13.2 eV above the ground state indicates that at least this much energy is available for the excitation process. Finally, there is no evidence of a background continuum. The random dots in Fig. 5a-e adjacent to the spectral lines are caused by Johnson noise in the image intensifier system.

Figure 5b is the spectrum produced by the fracture of Salida Granite in helium. The apparent broadening of the 7,065-Å line in the top reference spectrum was caused by overexposure. As in the case of argon, this spectrum shows only neutral atomic transitions. The upper state of the 7,065-Å transition lies 22.7 eV above the ground state. There is no evidence of a background continuum.

Figure 5c is the spectrum of Table Mountain Basalt (no piezoelectric minerals) in argon. This spectrum is similar in every feature to that produced by the failure of Salida Granite in argon. Again, the 7,635-Å line is prominent, and no lines are present other than those of the argon spectrum. There is no evidence of a continuum background.

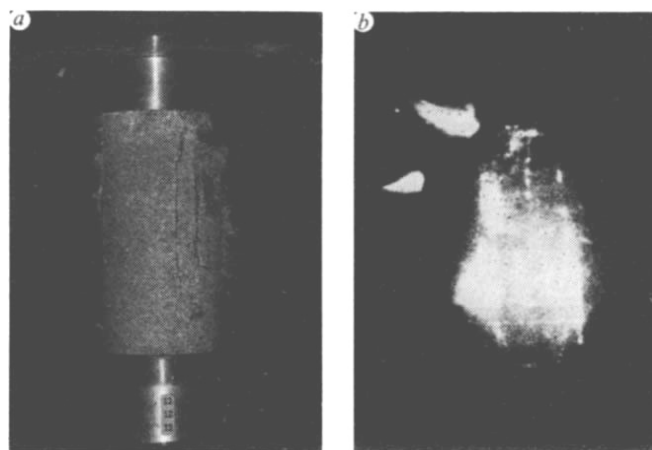


Fig. 4 a, Photograph of Salida Granite during failure. Maximum load at failure was 300 MPa. Load during photograph was 205 MPa. b, Light emission from failing Salida Granite. Note discrete emissions along failure surfaces. Samples shown in a and b are different.

Fig. 5 *a*, Spectrum from Salida Granite fracturing in argon atmosphere. Top spectrum is the reference gas spectrum. *b*, Spectrum from Salida Granite fracturing in helium atmosphere. *c*, Spectrum from Table Mountain Basalt fracturing in argon atmosphere. *d*, *e*, Spectra from Salida granite fracturing in water. Top reference spectra are $H\alpha$. *f*, Spectrum produced by frictional heating of two pieces of bulk quartz in air atmosphere. The reference line (5,460 Å) appears at top. No image intensifier used.

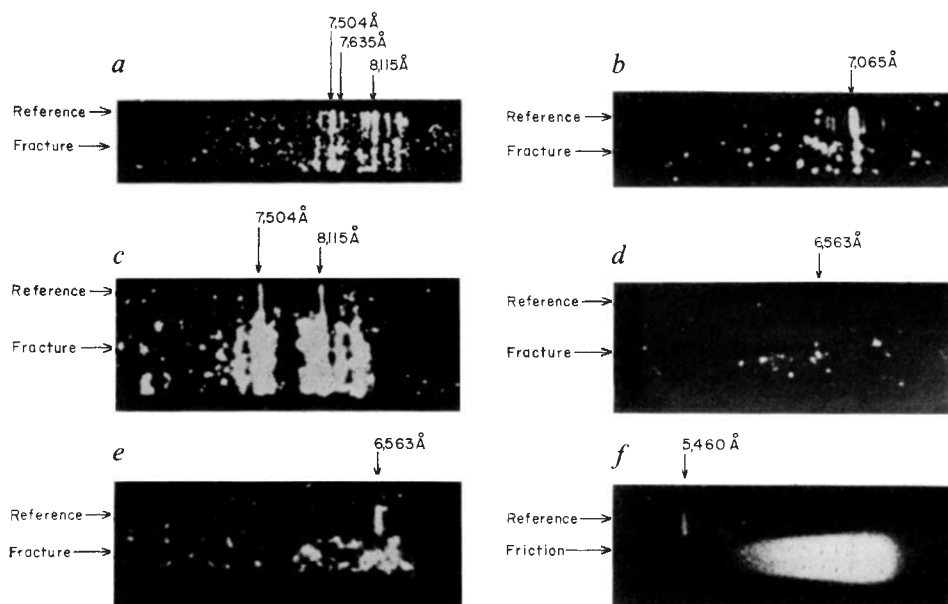


Figure 5*d* and *e* are spectra from Salida Granite being fractured in water. The single line in the top reference spectrum is hydrogen α ($H\alpha$), 6,563 Å (12.1 eV). The spectra have several important features. First, as seen in Fig. 5*d* and, in particular, visually through the spectrometer, radiation occurring at the 6,563-Å position can be positively identified as being $H\alpha$, produced by atomic hydrogen. In Fig. 5*e*, radiation centred around the $H\alpha$ position has been broadened into a band. This spectral broadening is attributed to perturbation of the atomic hydrogen energy levels by potential interactions in the liquid water. A second band of radiation extends from $\sim 5,700$ to 6,200 Å. This is identified as unresolved structure of the molecular hydrogen spectrum¹³. No other transitions are observed, and no background continuum is present. Thus, the spectra from Salida Granite being fractured in water indicate that both atomic and molecular hydrogen are produced during fracture.

Figure 5*f* is the spectrum produced from rubbing (frictionally heating) two pieces of quartz in air. No image intensifier was used in this experiment. A mercury reference spectrum appears at the top, with the 5,460-Å line prominent. The spectrum is a continuum. It is characteristic of a black-body radiator at a temperature of $\sim 1,500$ K, inferred from the spectral energy distribution. No line or band features are observed.

Regrettably, the photographic spectra from the fracture of Salida Granite in 10^{-6} torr vacuum are even less clear than those already discussed; therefore, only the results of visual observation are reported. The spectra observed exhibited typical molecular band structure. Comparison of the best photographic spectrum with that produced by a 10^{-3} torr air Geissler tube indicated that the spectrum was that of air. No line structure was detected visually or photographically and, consistent with the other experiments, no continuum radiation was observed.

Figure 6 is a photograph of the light emissions from fracturing a core of Salida Granite in an air atmosphere. The photograph was taken with ASA 400 film by turning out the room lights, opening the camera shutter, breaking the core, and then closing the shutter. The light source was determined to result from burning chips of steel scoured from the steel platens by the exploding rock fragments. The spectrum produced from this light is a continuum representative of oxidizing steel. That the light source was oxidizing steel was demonstrated in two ways. First, an increase in the oxygen content of the ambient atmosphere dramatically increased the light intensity, and second, by covering the upper platen with a thin piece of rubber, this

comparatively bright light disappeared, even in a 50% oxygen atmosphere.

Discussion

An important and surprising result of this investigation is the complete lack of spectroscopic evidence for continuum radiation. Continuum radiation would be produced by material heated to incandescence by frictional sliding. That such a continuum is produced by frictionally heated rock material and is easily detectable is confirmed by Fig. 5*f*. Other experiments were performed in which drill cores of various rock types were lightly ground together, and similar continuum radiation spectra were produced. The lack of continuum radiation from fracturing rock indicates that frictional heating does not produce the light observed during fracture and provides corroborative evidence of the explosive character of fracture shown in Figs 1 and 4*a*. Furthermore, the lack of continuum radiation also indicates that the finely ground dust material produced during rock fracture is not formed by frictional sliding, as is commonly believed. Microscopic examination of the rock dust shows euhedral surfaces with no apparent smoothing or roughening characteristic of frictional grinding. Thus, our results suggest a tensional origin for these fine-grained particles, such as could be produced by shock waves propagating through the rock structure during catastrophic failure.

An additional feature common to all recorded spectra is the absence of emission lines from ionized species. The absence of both continuum radiation and emission lines from ionized species eliminates the possibility that a plasma is a source of emitted light. However, note that the most significant argon (II) lines are located towards the ultraviolet end of the visible spectrum.

The absence from all the spectra of lines produced by the elemental constituents of the rock material is significant. Although the strongest silicon lines are in the ultraviolet region¹³, highly sensitive calcium, sodium and potassium lines occur in the visible and would have been readily detectable had they been present. There is no evidence of excitation of the constituent rock minerals. The spectra in all cases are those of the ambient atmosphere.

The piezoelectric excitation model^{1,3,7} has been seriously considered as a light-producing agent because many rock materials contain minerals known to be piezoelectric. In terms of an excitation mechanism, a piezoelectric discharge is best charac-

terized as spark-like, that is, a high-voltage differential with low current in which the excitation is due primarily to a high electric field. No ionized line spectra, such as typically produced by a spark discharge, were detected. The spectrum of basalt broken in an argon atmosphere is of interest in this case. Petrographic analysis confirmed that the basalt was free from any strongly piezoelectric minerals. Yet the spectrum of basalt in argon is identical to that produced by granite in argon. While deformation of piezoelectric minerals does occur during fracture of rocks, our evidence is that a piezoelectric mechanism is not responsible for the observed excitation of the ambient atmosphere. Finally, the absence of microwave emanations during the time interval in which the light is observed confirms the observation that a piezoelectric discharge is not the source of visible light¹¹.

Although most of our experimental evidence is indirect, there is substantial support for an excitation mechanism entailing electron bombardment. The tendency of a particle to produce ionizing events is a function of the particle's energy, mass and charge. An α -particle will typically produce 10^3 times more ionizing events in a medium than a β -particle of the same energy. The absence of spectra from ionized species argues against the excitation of the ambient atmosphere by α -particles or heavier nuclei. The fracture of Salida Granite in water produced both atomic and molecular hydrogen. The formation of these gases is similar to their generation when water is irradiated by β -particles (electron bombardment).

We conclude that exoelectron excitation of the ambient atmosphere is the mechanism responsible for the light emissions observed during rock fracture. For this mechanism to explain light emissions produced during massive failures in mines or earthquakes, it would be necessary for the fracture to reach the surface to allow exoelectron excitation of the ambient atmosphere. Recent observations have shown that light emissions, coincident with acoustic emissions resulting from rock spalling off the surface, have occurred along highly stressed drifts on the 5,400-ft level of the Consolidated Silver venture near Osburn, Idaho (J. Suveg, personal communication). Here the light emission is probably due to exoelectrons emitted from fresh fracture surfaces exciting the air. Of course, for this mechanism to be a source of light when the fracture does not reach the surface, as in deep crustal earthquakes where surface cracking does not occur, would require that sub-surface stress changes induced by the earthquake preparation process cause cracks on the surface which could then emit electrons. Note that surface cracking was observed in earthquakes where light emissions were reported, such as the 1965 Matsushiro earthquake swarm^{15,16} and the 1976 Tangshan earthquake (T. K. Tan, personal communication). Resolution of the light problem in mines and earthquakes will require spectroscopic analysis. An exoelectron excitation type of illumination is not likely to cause isolated light sources to appear several hundred metres above the Earth's surface, as is reported to occur in some earthquake-prone regions.

Last, our study shows that energy levels of the observed atomic and molecular transitions indicate a minimum of 23 eV is available from the exoelectron excitation mechanism. The molecular dissociation of water by rock fracture producing atomic and molecular hydrogen suggests that fracture might produce interesting chemical effects within a fluid or gas-saturated rock mass. For example, we speculate that exoelectron emission occurring

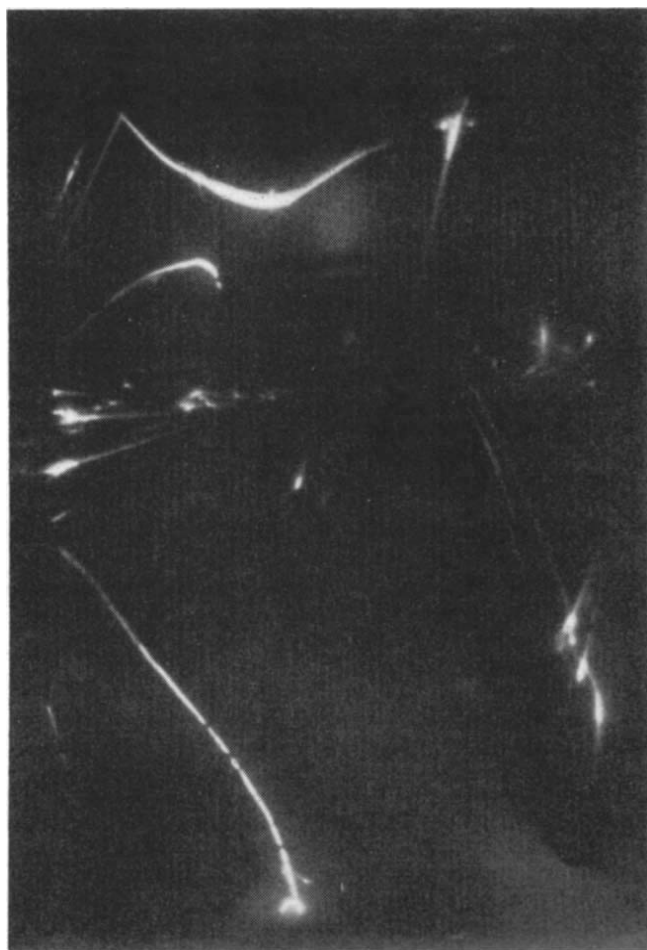


Fig. 6 Light emissions (discrete) from failing Salida Granite in air. Steel platens used in test. Light emissions are produced by oxidation of steel particles abraded from platens by high-velocity ($v = 2 \times 10^3 \text{ cm s}^{-1}$) rock dust.

within fracturing coal or potash rock masses may result in dissociation of methane, thereby producing hydrogen gas which could, in suitable conditions, be of importance in igniting a gas outburst. We believe that insufficient consideration has been given to the role of rock fracture in fluid (H_2O) and gas (CH_4 , CO_2 , H_2S , and so on) saturated rock masses in promoting molecular dissociations by exoelectrons and the role of this process in initiating chemical reactions of geological and biological interest. This problem needs to be thoroughly investigated.

We thank Stuart Hoenig, Tom Dickinson, John Derr, Michael Persinger, and Glynn Cress for conversations; Glen and Karen Winters (Winter Productions, Los Angeles, California), for exciting our interest in examining light emissions from fracturing rock and their role in explaining the occurrence of such emissions before earthquake-related phenomena; and last the research staff at the Denver Research Center for aid with the difficult experimental procedures.

Received 11 November 1985; accepted 12 March 1986.

1. Derr, J. *Bull. seism. Soc. Am.* **63**, 2177-2187 (1973).
2. Derr, J. & Persinger, M. A. *Experientia* (in the press).
3. Dmowska, R. *Geophys. Surv.* **3**, 157-174 (1977).
4. Paul, W. *Mining Lore* (Morris Printing Co., Portland, 1970).
5. Hoenig, S. A. & Cole, M. A. preprint, Univ. Arizona (1980).
6. Lockner, D. A., Johnston, M. J. S. & Byerlee, J. D. *Nature* **302**, 28-33 (1983).
7. Powell, J. R. & Finkelstein, D. *Am. Scient.* **58**, 262-280 (1970).
8. Dickinson, J. T., Donaldson, E. E. & Park, M. K. *J. Mater. Sci.* **16**, 2897-2908 (1981).

9. Hoenig, S. A. & Itani, F. S. *4th int. Congr. on Rock Mechanics*, Montreux, Switzerland (1979).
10. Parkhomenko, E. U. *Electrification Phenomena in Rocks* (Plenum, New York, 1971).
11. Cress, G. O., Brady, B. T. & Rowell, G. A. *Geophys. Res. Lett.* (submitted).
12. Wollbrandt, J., Linke, E. & Meyer, K. *Phys. stat. Sol.* **A27**, K53-K55 (1975).
13. Pearce, R. W. B. & Gaydon, A. G. *The Identification of Molecular Spectra* (Wiley, New York, 1941).
14. Weast, R. C. (ed.) *Handbook of Chemistry and Physics*, 53rd edn (Chemical Rubber Co., Cleveland, 1972-73).
15. Hamada, K. & Hagiwara, T. *Bull. Earthq. Res. Inst.* **44**, 1239-1268 (1966).
16. Nakamura, K. & Tsurunishi, Y. *Bull. Earthq. Res. Inst.* **44**, 1371-1384 (1966).

Isolation of the *paired* gene of *Drosophila* and its spatial expression during early embryogenesis

François Kilchherr, Stefan Baumgartner, Daniel Bopp, Erich Frei & Markus Noll

Department of Cell Biology, Biocenter of the University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland

We have cloned the paired gene of Drosophila melanogaster, a pair-rule gene required for the establishment of proper segmentation. The transcriptional pattern in young embryos shows developmental polarities along the antero-posterior and dorso-ventral axes. Transcripts, initially expressed with a double-segment periodicity, switch to a single-segment repeat during syncytial blastoderm.

SEGMENTATION, an important process of animal development, is the most conspicuous event of compartmentalization. A systematic search for mutations affecting segmentation in the fruitfly *Drosophila* has revealed that it is governed by ~30 genes which can be divided into four classes according to their mutant phenotypes^{1,2}. In the first class, the gap mutants, various overlapping non-terminal subregions of the larva are deleted. The second class contains the segment-polarity mutants which exhibit defects that are reiterated in every segment; whereas in the third class, the pair-rule mutants, structures are deleted at a two-segment periodicity (Fig. 1). These three classes of mutants suggest that segmentation involves three different units of spatial organization: one of a single-segment periodicity; one of a two-segment periodicity and a third comprising groups of adjacent segments and exhibiting no obvious periodicity. Mutants of the fourth class, the coordinate mutants, are all maternal and show global effects on the segmentation pattern. Their phenotypes have been explained by a defect in the expression of maternal components that regulate the genes of the three other classes^{1,2}.

In *Drosophila*, segmentation becomes visible as early as 1 h after the onset of gastrulation¹. Even earlier, however, during cellular blastoderm, cell lineage may already become restricted to segments³⁻⁷. At the molecular level, this is probably reflected by the spatially restricted expression of the pair-rule gene *fushi tarazu* (*ftz*) at cellular blastoderm, which shows a repetitive pattern of seven evenly spaced bands⁸ that correspond to the primordia of the regions deleted at a two-segment periodicity in homozygous *ftz* mutants^{1,2}. More recently, another pair-rule gene, *hairy* (*h*), has been cloned^{9,10}. Its expression, as analysed by *in situ* hybridization to blastoderm sections, exhibits a pattern of the same periodicity as *ftz* but is shifted anteriorly with respect to *ftz*, by slightly more than one segment primordium¹⁰. In addition, *h* transcripts are found in a band of the anterior dorsal blastoderm, extending from 85 to 95% of the egg length (EL)¹⁰.

Here we report the isolation of the pair-rule gene *paired* (*prd*) whose mutant phenotype is shown in Fig. 1. Transcripts of *prd* exhibit a pattern of seven evenly spaced bands during late syncytial blastoderm, reflecting a double-segment periodicity similar to the previously analysed pair-rule genes *ftz* and *h*. However, in contrast to the patterns of *ftz* and *h*, this pattern of *prd* transcripts is expressed only transiently and changes during cellularization of the blastoderm to a pattern with a single-segment repeat length of 14 regularly spaced bands. In addition, *prd* transcripts are detected in a dorsal band close to the anterior pole at a position similar to that seen for *h* transcripts¹⁰ and corresponding to 87 to 93% of EL.

Chromosomal walk from *esc* to *prd*

Recently, we cloned and identified the *extra sex combs* (*esc*) gene from DNA sequences at the cytogenetic locus 33B1,2 on the left arm of chromosome 2 (refs 11, 12). The initial clones,

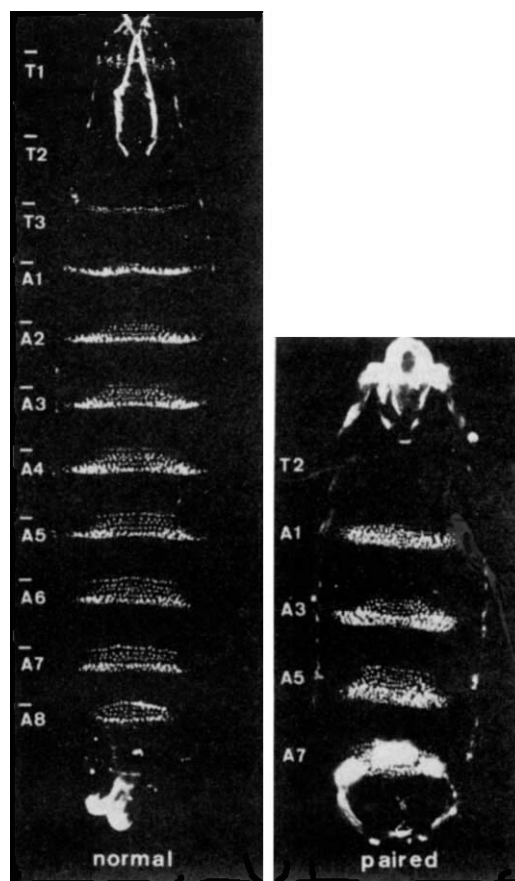


Fig. 1 Ventral cuticular pattern of a wild-type and a homozygous *prd*⁻ *Drosophila* larva. In the wild type the three thoracic (T) and eight abdominal (A) segments are indicated. In *prd*⁻, a pair-rule mutant, analogous portions of segments are deleted at a two-segment periodicity, giving rise to only half the normal number of segments¹. Because the missing portions include every other segment boundary, the segments are of a composite nature¹. For example, in the A1/A2 segment the anterior part is derived from A1, the posterior part from A2. Accordingly, the designation of segments in the *prd* mutant refers to the character of the anterior parts of the composite segments¹. Courtesy of C. Nüsslein-Volhard (from ref. 1).

obtained by microdissection of salivary gland chromosomes¹³, were spread over a region of 500 kilobase pairs (kb) comprising the entire deletion *Df*(2L)*esc*¹⁰. The most proximal clones, 1b.1 and 1b.8, were located just to the right of the deficiency *esc*¹⁰ and at the left end of a deficiency of *paired*, *Df*(2L)*prd*^{1,7,20} (ref. 1). The *prd* gene has been mapped previously by the deficiency

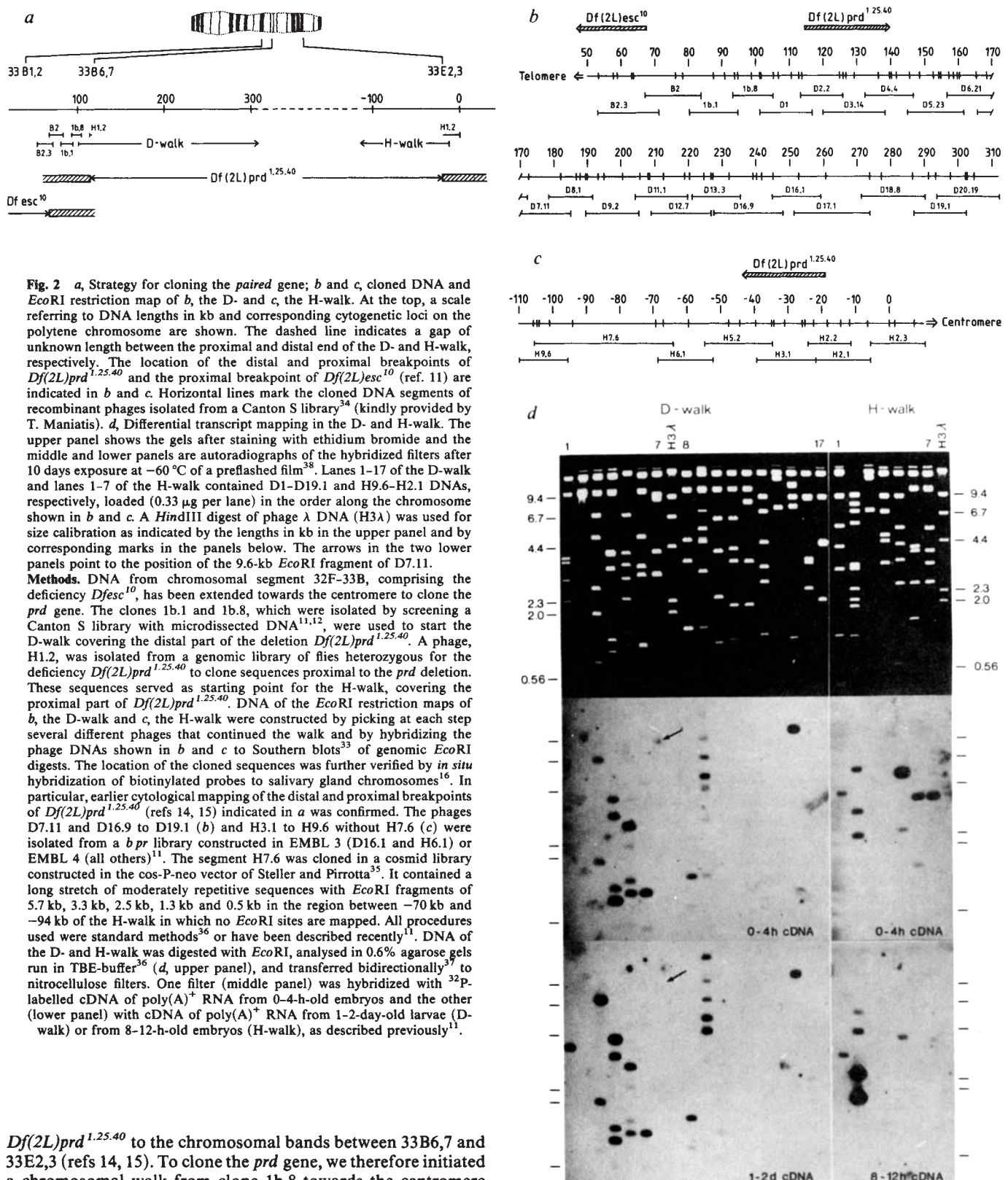


Fig. 2 *a*, Strategy for cloning the *prd* gene; *b* and *c*, cloned DNA and *EcoRI* restriction map of *b*, the D- and *c*, the H-walk. At the top, a scale referring to DNA lengths in kb and corresponding cytogenetic loci on the polytene chromosome are shown. The dashed line indicates a gap of unknown length between the proximal and distal end of the D- and H-walk, respectively. The location of the distal and proximal breakpoints of *Df(2L)prc1.25.40* and the proximal breakpoint of *Df(2L)esc¹⁰* (ref. 11) are indicated in *b* and *c*. Horizontal lines mark the cloned DNA segments of recombinant phages isolated from a Canton S library³⁴ (kindly provided by T. Maniatis). *d*, Differential transcript mapping in the D- and H-walk. The upper panel shows the gels after staining with ethidium bromide and the middle and lower panels are autoradiographs of the hybridized filters after 10 days exposure at -60 °C of a preflashed film³⁸. Lanes 1-17 of the D-walk and lanes 1-7 of the H-walk contained D1-D19.1 and H9.6-H2.1 DNAs, respectively, loaded (0.33 µg per lane) in the order along the chromosome shown in *b* and *c*. A *HindIII* digest of phage λ DNA (H3λ) was used for size calibration as indicated by the lengths in kb in the upper panel and by corresponding marks in the panels below. The arrows in the two lower panels point to the position of the 9.6-kb *EcoRI* fragment of D7.11. **Methods.** DNA from chromosomal segment 32F-33B, comprising the deficiency *Df(2L)esc¹⁰*, has been extended towards the centromere to clone the *prd* gene. The clones 1b.1 and 1b.8, which were isolated by screening a Canton S library with microdissected DNA^{11,12}, were used to start the D-walk covering the distal part of the deletion *Df(2L)prc1.25.40*. A phage, H1.2, was isolated from a genomic library of flies heterozygous for the deficiency *Df(2L)prc1.25.40* to clone sequences proximal to the *prd* deletion. These sequences served as starting point for the H-walk, covering the proximal part of *Df(2L)prc1.25.40*. DNA of the *EcoRI* restriction maps of *b*, the D-walk and *c*, the H-walk were constructed by picking at each step several different phages that continued the walk and by hybridizing the phage DNAs shown in *b* and *c* to Southern blots³³ of genomic *EcoRI* digests. The location of the cloned sequences was further verified by *in situ* hybridization of biotinylated probes to salivary gland chromosomes¹⁶. In particular, earlier cytological mapping of the distal and proximal breakpoints of *Df(2L)prc1.25.40* (refs 14, 15) indicated in *a* was confirmed. The phages D7.11 and D16.9 to D19.1 (*b*) and H3.1 to H9.6 without H7.6 (*c*) were isolated from a *bpr* library constructed in EMBL 3 (D16.1 and H6.1) or EMBL 4 (all others)¹¹. The segment H7.6 was cloned in a cosmid library constructed in the cos-P-neo vector of Steller and Pirrotta³⁵. It contained a long stretch of moderately repetitive sequences with *EcoRI* fragments of 5.7 kb, 3.3 kb, 2.5 kb, 1.3 kb and 0.5 kb in the region between -70 kb and -94 kb of the H-walk in which no *EcoRI* sites are mapped. All procedures used were standard methods³⁶ or have been described recently¹¹. DNA of the D- and H-walk was digested with *EcoRI*, analysed in 0.6% agarose gels run in TBE-buffer³⁶ (*d*, upper panel), and transferred bidirectionally³⁷ to nitrocellulose filters. One filter (middle panel) was hybridized with ³²P-labelled cDNA of poly(A)⁺ RNA from 0-4-h-old embryos and the other (lower panel) with cDNA of poly(A)⁺ RNA from 1-2-day-old larvae (D-walk) or from 8-12-h-old embryos (H-walk), as described previously¹¹.

Df(2L)prc1.25.40 to the chromosomal bands between 33B6,7 and 33E2,3 (refs 14, 15). To clone the *prd* gene, we therefore initiated a chromosomal walk from clone 1b.8 towards the centromere (shown as the D-walk in Fig. 2a). Crossing the distal breakpoint of the *Df(2L)prc1.25.40* deletion allowed us to clone the DNA fragment H1.2, containing sequences from both sides of the deletion (Fig. 2a), by screening a genomic library constructed from flies heterozygous for the deficiency *Df(2L)prc1.25.40*. From proximal sequences of the clone H1.2, a second walk was then started into the *Df(2L)prc1.25.40* deletion (shown as the H-walk in Fig. 2a). The D- and H-walks are shown in Fig. 2b, c. Together they cover ~300 kb of the *Df(2L)prc1.25.40* deletion.

Screening for the *prd* gene

Although the converging sequences of the D- and H-walk had not joined, a screen for possible candidates of the *prd* gene by differential transcript mapping was carried out, similar to the screen that proved successful when the *esc* gene was identified^{11,12}. Radioactively labelled complementary DNAs of poly(A)⁺ RNA isolated from egg follicles, 0-4- and 8-12-h-old

embryos and 1–2-day-old larvae were hybridized to Southern blots of *EcoRI* digests of the D- and H-walk (Fig. 2d). We screened for DNA fragments that were labelled with cDNA from 0–4-h-old embryos (middle panel of Fig. 2d) but remained unlabelled when hybridized to cDNA from later stages of development (lower panel of Fig. 2d) or egg follicles (not shown). This expectation was based (1) on experiments with a temperature-sensitive *prd* allele showing zygotic expression during the blastoderm stage¹ and (2) on the absence of a maternal effect of the *prd* gene¹⁵. In the D-walk, only one such fragment was found, a 9.6-kb *EcoRI* fragment of phage D7.11 (arrows in Fig. 2d). In the H-walk, two regions at the proximal end, the 3.9-kb *EcoRI* fragment of H2.2 and the 2.6-kb *EcoRI* fragment of H3.1 (which comprises the 1.6-kb left terminal fragment of H2.2) were transcribed in 0–4-h-old embryos but not in 8–12-h-old embryos (Fig. 2d). However, these fragments of the H-walk are unlikely to harbour the *prd* gene because they are also heavily labelled by cDNA of poly(A)⁺ RNA from follicles (data not shown). Therefore, if the *prd* gene was located within the 300 kb of the *Df(2L)prd*^{1,25,40} that had already been cloned, the most likely location was the 9.6-kb *EcoRI* fragment of D7.11, at about 170–180 kb in the D-walk or 240 kb to the right of the *esc* gene^{11,12}.

Mapping the mutant *prd*^{2.45.17}

To test whether the *prd* gene was contained within the cloned 300 kb, and specifically within the 9.6-kb *EcoRI* fragment of D7.11, the D- and H-walk DNA of an X-ray-induced *prd* mutant, *prd*^{2.45.17}, was compared with that of its parent strain by whole genome Southern analysis of *EcoRI* digests. No differences were detected except for a 1.1-kb insertion into the 9.6-kb *EcoRI* fragment of D7.11 in the *prd*^{2.45.17} mutant (data not shown). The mutated DNA was isolated from a λ phage library of the heterozygous *prd*^{2.45.17} mutant, and the 4.0-kb *BamHI* fragment containing the 1.1-kb insert was subcloned. We compared its restriction map with that of the corresponding 2.9-kb *BamHI* fragment of the *prd*⁺ DNA to locate the insertion more precisely. As shown in Fig. 3, in *prd*^{2.45.17} a 1.1-kb DNA sequence has been inserted into a 0.41-kb *SmaI* fragment of the *prd* DNA. When a biotinylated probe consisting of the 1.5-kb *SmaI* fragment from *prd*^{2.45.17} (Fig. 3) was hybridized to salivary gland chromosomes¹⁶ of an Oregon R strain, it labelled more than 100 bands scattered over all chromosome arms (not shown).

Developmental profile of the *prd*⁺ transcript

Although both differential transcript mapping as well as whole genome Southern analysis suggested that the *prd* gene was located in D7.11, the possibility remained that a gene with a developmental profile similar to that of *prd* was present in D7.11 and that the 1.1-kb insert was fortuitous and did not result in an altered gene expression at its location. This objection was excluded by Northern analysis of the *prd*^{2.45.17} mutant in this region. As shown in Fig. 4, two poly(A)⁺ RNAs of 2.5 and 3.6 kb were detected in embryos of heterozygous *prd*^{2.45.17} parents whereas only a single RNA species of 2.5 kb was found in *prd*⁺ embryos. Hence, the insert in the mutant DNA is located within a transcript that exhibits the developmental profile expected for the *prd* gene. The *prd* mRNA, while absent in oocytes, accumulates to a sharp peak in 2–4-h-old embryos (blastoderm and early gastrula), followed by a precipitous decline (Fig. 4). Essentially no 2.5-kb transcripts were detectable after 12 h of embryonic development (stages after 18 h are not shown) which is consistent with the results obtained with a temperature-sensitive allele of the *prd* gene¹.

Spatial transcription pattern

Genetic analysis¹ and the developmental profile shown in Fig. 4 suggest that the main period of *prd*⁺ activity occurs around cellular blastoderm. In addition, inspection of the cuticular pattern of homozygous *prd*[−] larvae (Fig. 1) reveals that the

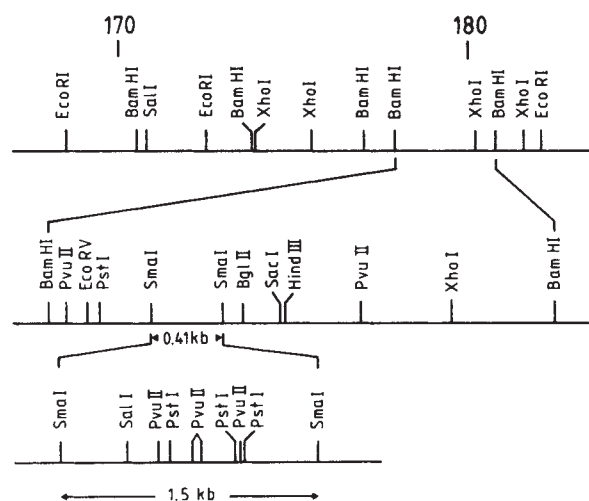


Fig. 3 Restriction map of DNA containing the *paired* gene and of *prd*^{2.45.17} mutant DNA. A restriction map of the *paired* region, obtained from D7.11, is shown at the top. Below, the 2.9-kb *BamHI* fragment, subcloned from D7.11, has been mapped in more detail. At the bottom a restriction map of the *prd*^{2.45.17} mutant DNA, containing the 1.1-kb insertion of a repetitive element, is illustrated. This region was mapped by isolating the mutated DNA from a *prd*^{2.45.17}/*prd*⁺ library according to standard methods³⁶ and mapping a subclone of the corresponding 4.0-kb *BamHI* fragment containing the inserted DNA.

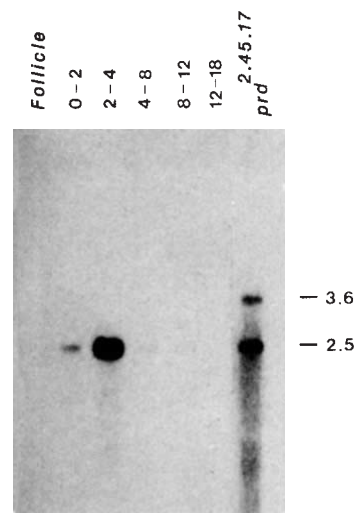


Fig. 4 Northern analysis of the *prd*⁺ and *prd*^{2.45.17} transcripts. For size calibration, end-labelled fragments of a partial *RsaI* digest of pBR322 (4.36 kb, 3.68 kb, 2.80 kb, 2.25 kb, 2.12 kb, 1.57 kb and 0.68 kb) were used (data not shown).

Methods. Poly(A)⁺ RNA (2 μ g each), isolated from wild-type egg follicles and 0–2-h, 2–4-h, 4–8-h, 8–12-h and 12–18-h-old embryos and from 0–4-h-old embryos of heterozygous *prd*^{2.45.17}/*prd*⁺ parents, as previously described¹¹, were run in a 1.1% agarose gel containing formaldehyde³⁹. The RNA was transferred in 20 $\times$ SSC to a nitrocellulose filter according to Southern³³ and hybridized with a cDNA fragment, c73.2, nick-translated⁴⁰ with [α -³²P]dATP (3,000 Ci mmol^{−1}) and consisting of 400 base pairs (bp) at the 3' end of the *prd*⁺ transcript located to the immediate left of the 0.9-kb *BamHI* fragment (Fig. 3). The same 2.5-kb RNA was observed after hybridization with the genomic 0.9-kb *BamHI* fragment and with the genomic fragments to its left (*XhoI*–*BamHI*) and right (*PvuII*–*PvuII*), whereas no RNA was detected in a region of at least 2 kb to the right of the *XhoI* site at 180 kb (Fig. 3). A 2.3-kb cDNA clone, c7340.6, isolated from a cDNA library constructed from poly(A)⁺ RNA of 0–4-h-old embryos, spanned the same region, labelling only DNA within the 4.7-kb *XhoI* fragment between 175 and 180 kb on the genomic map (Fig. 3).

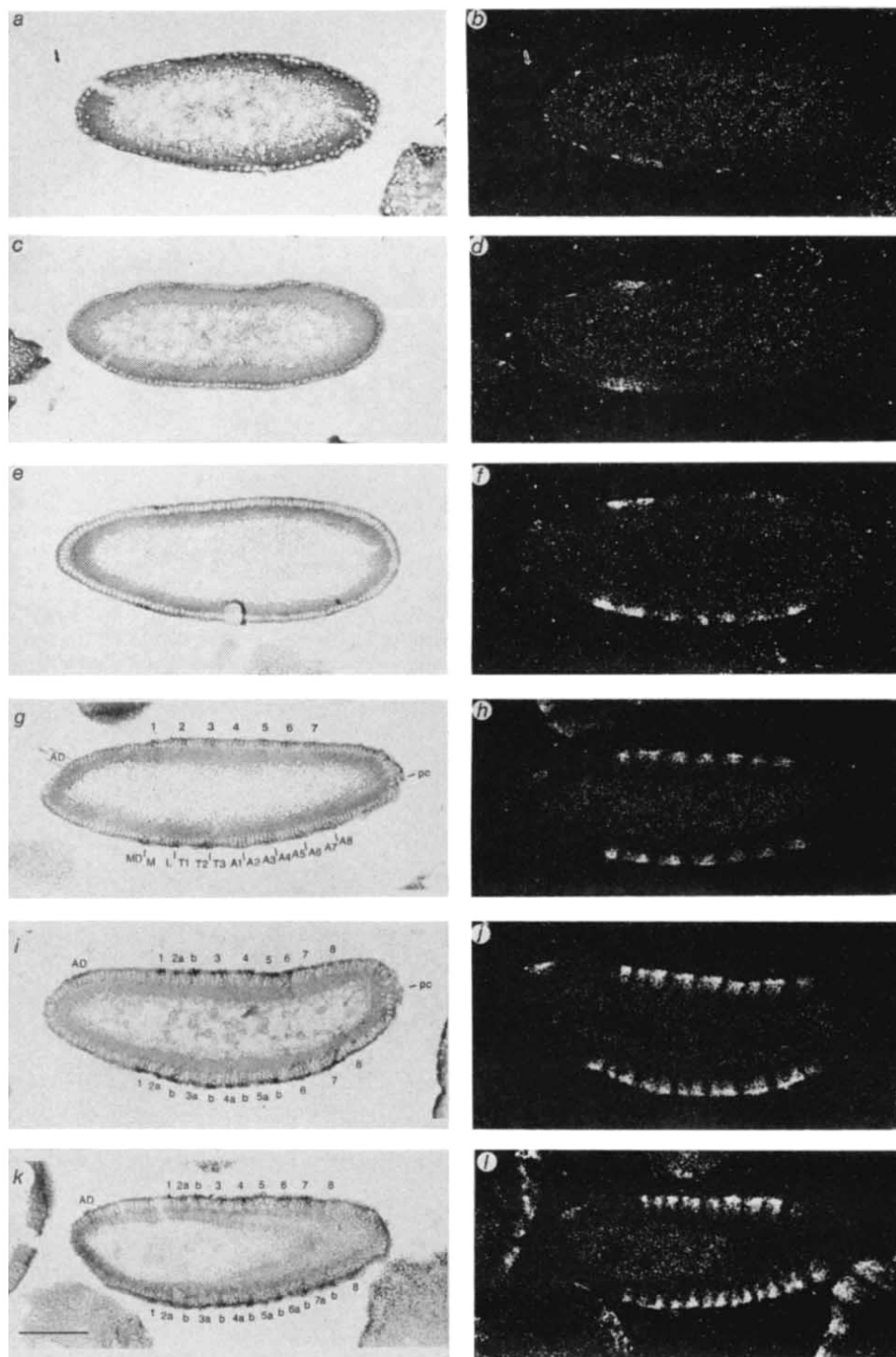


Fig. 5 Localization of *prd* transcripts in tissue sections of embryos at syncytial and cellular blastoderm. The panels show photomicrographs of the same embryos taken under phase contrast (left) or dark-field illumination (right). Sagittal or parasagittal sections through embryos during the 12th (a,b), 13th (c,d) and 14th nuclear cycle (e-f) of syncytial blastoderm, or at cellular blastoderm (g, i), are shown. Tissue sections are oriented with their dorsal side up and their anterior end to the left. The orientation was determined by morphological markers such as pole cells (pc) visible in other sections exhibiting very similar labelling. The presumptive locations of thoracic (T1-T3), abdominal (A1-A11), as well as labial (L), maxillary (M), and mandibular (MD) head segment primordia are indicated. The bands are labelled AD (anterior dorsal), 1-7 at early, and 1a-7b and 8 at late cellular blastoderm. The horizontal bar in k indicates a length of 0.1 mm. **Methods.** Tissue sections through young embryos were prepared, hybridized *in situ* with ^3H -labelled 2.2-kb *prd* cDNA, c7340.1 and exposed according to Hafen *et al.*⁴¹. Autoradiographic exposure was for 29 days except in f, which shows a 20-day exposure.

equivalent of one segment is missing for every two segments^{1,2}. From previous studies on the pair-rule genes *fiz* (ref. 8) and *h* (ref. 10), in which the structures deleted in the mutants correspond approximately to the wild-type primordia in which the genes are transcribed, we would expect *in situ* hybridizations of embryonic tissue sections with ^3H -labelled *prd*-cDNA to show a double-segment periodicity expressed at cellular blastoderm. Surprisingly, we do not observe such a strict correlation between the mutant phenotype of *prd* and the spatial distribution of its transcripts in the wild-type embryo (Fig. 5).

Localized *prd* transcripts are first observed during syncytial blastoderm (nuclear cycle 12; ref. 17) in a band 6 nuclei wide which is stronger on the ventral than on the dorsal side and

located at a distance of approximately 13 nuclei from the anterior end; that is, between 63 and 77% of EL (Fig. 5a, b). During the following nuclear cycle, the band widens to ~12-16 nuclei on the ventral and 8-10 nuclei on the dorsal side (Fig. 5c, d). It is separated by ~16-20 nuclei from the anterior pole and thus extends between 60 and 75% of EL, corresponding to the location of the primordial head segments^{6,18}. The nuclei in the anterior portion of the band are labelled more strongly than those in the posterior half. The transcripts clearly accumulate in the cortical part of the cytoplasm surrounding the nuclei, as has been observed previously for transcripts of *ftz* (ref. 8) and *h* (ref. 10). During the fourteenth nuclear cycle, at late syncytial blastoderm when nuclei begin to elongate, five additional bands

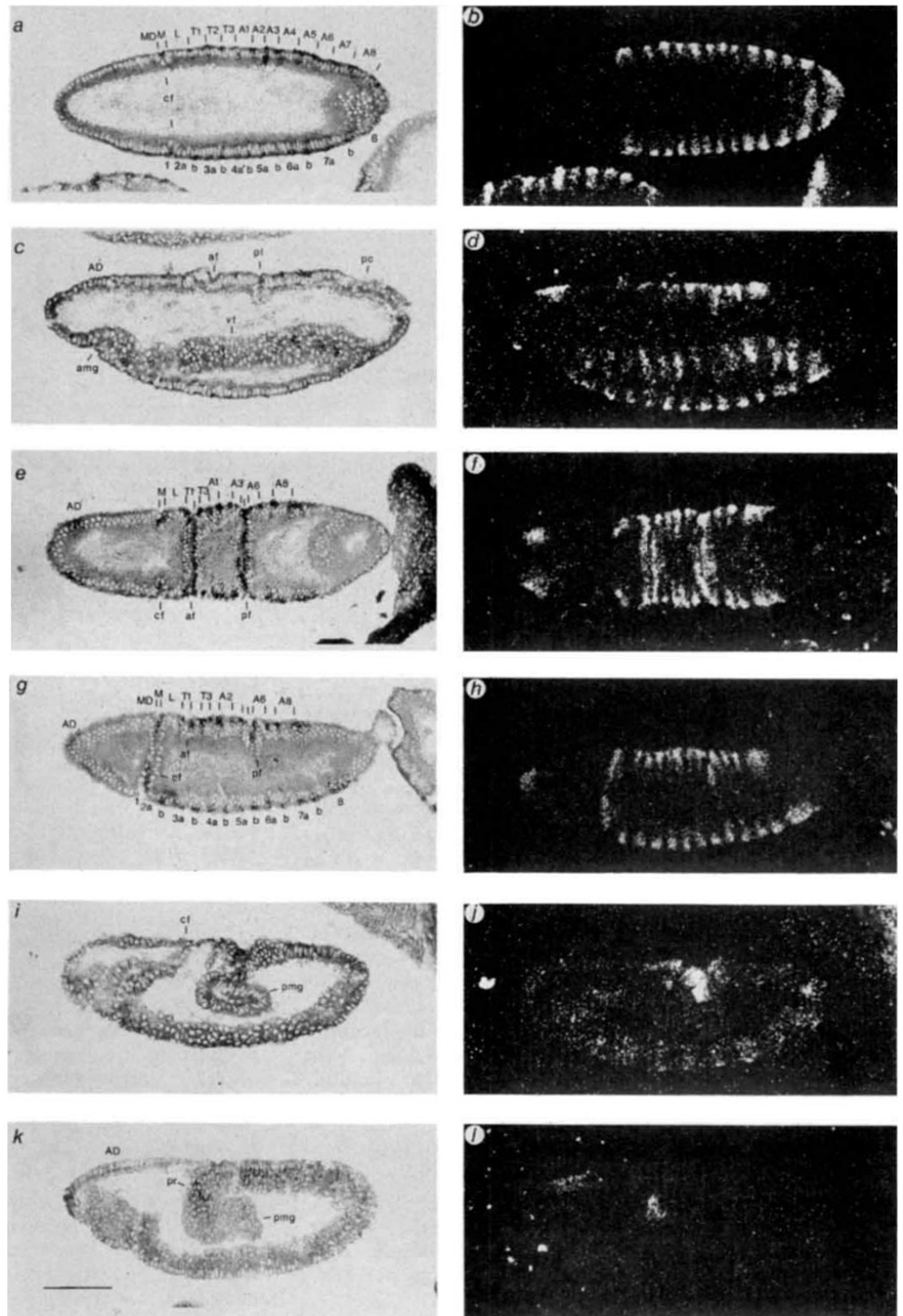


Fig. 6 Localization of *prd* transcripts in gastrulating embryos. Photomicrographs of embryonic tissue sections, prepared and treated as in Fig. 5, were taken under phase contrast (left) or dark-field illumination (right). The following developmental stages are shown: *a, b*, formation of the cephalic furrow; *c, d*, formation of the ventral furrow; *e-h*, beginning of germ-band extension at 3.5–4 h; *i, j*, germ-band extension at 4–4.5 h; *k, l*, end of germ-band extension at ~5 h development at 25 °C. The sections chosen were *a, b*, midventral and *e, f*, superficial dorsal horizontal sections; *c, d, g-l*, median or lateral sagittal sections. The anterior end is oriented to the left and, in sagittal sections, the dorsal side up. Autoradiographic exposure was for 20 days (*a-d, i-l*) or 29 days (*e-h*). The probes used for hybridization in Figs 5 and 6 comprise two short regions of the *prd* gene that are homologous with sequences elsewhere in the genome (G. Frigerio and M.N., unpublished results). However, the same patterns of hybridization are observed when a probe is used from which these *prd*-cDNA sequences have been deleted. Bands and primordial segments are labelled as in Fig. 5. The horizontal bar in *k* indicates a length of 0.1 mm. Abbreviations: af, anterior transverse fold; amg, anterior midgut; cf, cephalic furrow; pc, pole cells; pf, posterior transverse fold; pmg, posterior midgut invagination; pr, proctodeum.

of transcripts appear at approximately equal intervals. These bands are located posterior to the first band and extend to ~20% of EL, that is, to the eighth abdominal segment primordium^{6,18}. At the same time, the early anterior band splits into two bands equal in width to the five posterior bands (Fig. 5*e, f*). At this stage, therefore, *prd* transcripts occur in seven bands located between 20 and 75% of EL. The spacing of the bands is regular but closer on the dorsal than on the ventral side, and the signal intensity of the newly formed five posterior bands is again weaker dorsally than ventrally, indicating a dorso-ventral polarity of pattern development (Fig. 5*e, f*). These seven bands increase in intensity as the nuclei elongate and plasma membranes grow inwards (Fig. 5*g, h*). While the width of the posterior six bands

remains unchanged at 5–7 cells, the most anterior band is reduced in width to about four cells. This results from an elimination of transcripts in the cells of the anterior portion of the first band. In each of these six posterior bands, however, the labelling intensity is skewed in such a way that cells of the anterior half exhibit less *prd* transcripts than cells of the posterior half. The cells transcribing the *prd* gene are separated by gaps of ~2–3 cells in which no *prd* transcripts are detectable. At the same time transcripts begin to accumulate in cells located close to the anterior pole of the embryo, forming a band six cells wide (Fig. 5*g, h*) which extends between 87 and 93% of EL (Fig. 5*i, j*). In contrast to the other bands, this new band covers only the dorsal half of the embryo (Fig. 5*g-j*).

At a slightly later time, shortly before the completion of cellular blastoderm, two conspicuous events take place (Fig. 5i, j). First, an additional band (8) appears posterior to band 7, extending the banding pattern to ~13% of EL. Second, bands 2 to 7 begin to split into an anterior and a posterior band, a process that moves from the anterior to the posterior pole. At the stage shown in Fig. 5i, j, the splitting of band 2 is nearly complete whereas that of bands 6 and 7 is barely visible. This band splitting is accomplished by elimination of the transcripts in the two middle cells of each band. As a result of this process, band 2 is split into an anterior band 2a and a posterior band 2b, band 3 into 3a and 3b, and so forth. By the time of cellular blastoderm, the splitting of bands 2–7 is complete, and the width of band 1 has been further reduced from 4 to its two most posterior cells (Fig. 5k, l). Thus, when cellularization of the blastoderm is nearly complete, 13 bands have arisen that are about two cells wide and are separated by gaps of about two cells on the ventro-lateral side. Dorsally, splitting of the bands occurs later and is complete only in band 2 at the stage illustrated in Fig. 5k, l. In addition, the intensity appears to be skewed in that more transcripts accumulate in the posterior than in the anterior band. The most posterior band (8) remains unsplit and is about five cells wide. Thus, both the formation of the transcript pattern exhibiting a double-segment periodicity and its transition to one with a single-segment repeat are oriented in an anterior–posterior as well as in a ventral–dorsal direction.

Transcript distribution and morphology

To assess the morphological significance of these transcription patterns, we have attempted to relate the spatial distribution of the label to morphological features that are well known from microscope studies^{19–21}. This required an extension in time to gastrulation when such features are beginning to appear. Initially, gastrulation is marked by the formation of the ventral and cephalic furrows. As evident from Fig. 6a, b, the first two bands (1 and 2a) migrate into the anterior and posterior part of the cephalic furrow. In a similar manner, as the ventral furrow forms, giving rise to the mesodermal germ band, cells transcribing *prd* migrate into it. This is evident from the persistence of the banding pattern in register across the ventral furrow (Fig. 6c, d). Similarly, at a slightly later stage when the dorsal transverse folds appear, cells of bands coinciding with the locations of these folds move into them (Fig. 6e–h). At this stage, the dorsal portions of the bands have also completed their splitting (Fig. 6e–h). From the end of cellular blastoderm to this stage no dramatic change in band intensities is observed. However, during the next stage of germ-band extension, all bands fade with the exception of the anterior dorsal invagination (Fig. 6k, l) and the unsplit band 8, located at the protodermal invagination (Fig. 6i, j). The intensity with which these two bands persist is remarkable. Only after germ-band retraction, shortly before the beginning of head involution, have these bands disappeared (data not shown).

ftz transcripts and segment primordia

Previous determinations of the positions of the *ftz* bands in the blastoderm embryo^{8,22} allow us to identify the blastoderm primordia expressing the *prd* gene. When hybridizing three consecutive sections with probes for either *prd* or *ftz*, or with both probes, we found that the pattern of *prd* transcripts is shifted to the anterior of the *ftz* bands. Whereas band 1 of *prd* does not overlap with *ftz* signals, the anterior borders of bands 2–7 (2a–7a) and band 8 of *prd* coincide with the anterior borders of bands 1–7 of *ftz* (Fig. 7). As the *ftz* bands are only about four cells wide and are spaced by gaps of about four cells^{8,10,22,23}, cells between the seven *prd* bands at late syncytial blastoderm (Fig. 5g and h) also remain unlabelled by *ftz* (data not shown). At the later stage of cellular blastoderm, when the repetitive pattern of *prd* transcripts consists of 14 bands (Figs 5k, l; 7a),

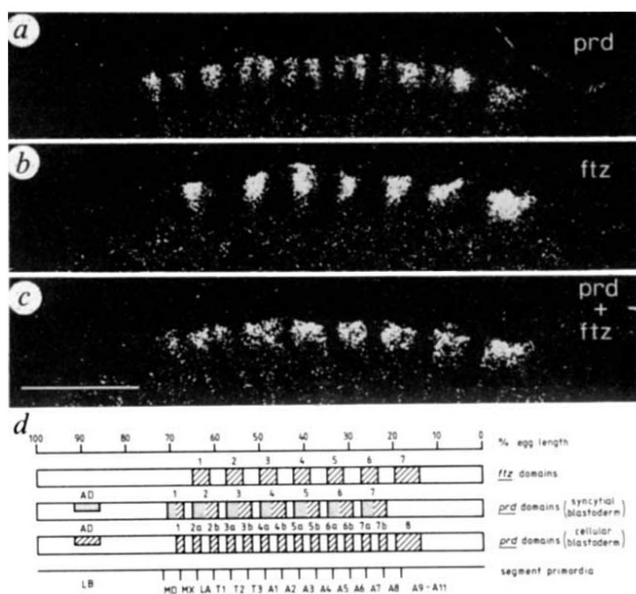


Fig. 7 Localization of *prd* relative to *ftz* transcripts. Three consecutive ventral horizontal sections through an embryo at cellular blastoderm were hybridized with *a*, ³H-labelled c7340.1 *prd* cDNA; *b*, p523B *ftz* DNA^{8,42,43}; *c*, the two probes combined and exposed for 29 days according to Hafen *et al.*⁴¹. Only one lateral side of the embryo is shown under dark-field illumination. The anterior end is oriented to the left. In *d*, the domains of *ftz* and *prd* expression are illustrated schematically relative to each other and the segment primordia. Stippled and hatched areas indicate blastoderm regions showing low and high numbers of transcripts, respectively. The *ftz* domains represented are those seen at syncytial blastoderm when they are about four nuclei wide and regularly spaced by gaps of about four nuclei^{8,22}. By the time of cellular blastoderm (*b*), *ftz* domains have been reduced in width to about three cells²². The most posterior *ftz* domain is broader and about six cells wide, as previously noted¹⁰. The horizontal bar in *c* indicates a length of 0.1 mm.

the cells of every second gap still express *ftz* (Fig. 7b). Thus, in the combined pattern of *prd* and *ftz* transcripts, we still observe a narrow anterior band two cells wide, consisting only of *prd* transcripts, and seven wide posterior bands, about six cells wide, separated by gaps of about two cells (Fig. 7c). Because the third *ftz* band covers the posterior compartment of the metathoracic and the anterior compartment of the first abdominal segment primordium^{8,22,24}, we conclude that band 4a of *prd* transcripts spans the T3/A1 segment boundary, as indicated in Figs 6a, b and 7d. If we assume that the four-cell repeat length of the *prd* banding pattern observed at this stage corresponds to the length of one segment primordium, we find that the most anterior *prd* band (1) crosses the boundary between the mandibular and maxillary segment primordia and that band 7b crosses the boundary between the seventh and eighth abdominal primordial segments (Figs 6a, b; 7d). Accordingly, the most posterior band (8) would cover the posterior compartment of A8 and probably the segment primordia of A9–A11 (Figs 6a, b; 7d). Our interpretation of the positions of the *prd* bands relative to those of *ftz* is confirmed by their relative positions with respect to the cephalic furrow. Whereas the most anterior band of *ftz* labels the posterior portion of the cephalic furrow⁸, bands 1 and 2a of *prd* are situated at its anterior and posterior edge (Fig. 6a, b, e–h).

Knowing how the banding pattern of *prd* RNA at early gastrula (Fig. 6a, b) develops from that at late syncytial blastoderm (Fig. 5g, h), we may trace back the location of *prd* transcripts with respect to the primordial segments at blas-

to derm. Thus, we find that the more strongly labelled posterior portions of bands 1–7 cross the boundaries between mandibular and maxillary, labial and first thoracic, second and third thoracic segment primordia, and so on, as indicated in Figs 5g, h, 7d. Similarly, we may identify segment primordia and relate them to morphological markers during later developmental stages by following the fate of cells labelled with *prd* transcripts. Accordingly, the prothoracic segment gives rise to the anterior transverse fold while the fourth and fifth abdominal segments coincide with the posterior transverse fold (Fig. 6e–h).

Discussion

Our analysis of the spatial distribution of *prd* transcripts during embryogenesis has revealed a more complex pattern than anticipated on the basis of results from previous studies with the pair-rule genes *ftz* (ref. 8) and *h* (ref. 10). Transcripts of *prd* appear first during the twelfth nuclear cycle in the region where the primordial head segments form. During subsequent nuclear divisions of the syncytial blastoderm, a pattern of seven bands builds up posteriorly along the antero-posterior axis and dorsally along the ventral–dorsal axis. At the end of this process, the *prd* pattern is similar to that of *ftz* (ref. 8) or *h* (ref. 10), showing a periodicity corresponding to two primordial segments. However, in contrast to the *ftz* and *h* patterns, the seven *prd* bands are wider and hence each covers more than one segment primordium. Thus, no simple correlation of the location of *prd* transcripts with the *prd* phenotype^{1,2} (Fig. 1) exists. To derive such a correlation, as has been possible for *ftz* (ref. 8) and *h* (ref. 10), it might be postulated that only those portions of segments are deleted in the mutant that exhibit strong transcription of the *prd* gene in the corresponding wild-type primordial segments during late syncytial blastoderm. As evident from Fig. 5g, h, these are the posterior parts of each of the seven *prd* bands, corresponding to the posterior mandibular plus anterior maxillary segment, posterior labial plus anterior first thoracic segment and so on (Fig. 7d). That this pattern of transcripts corresponds reasonably well to that of fused and deleted segments in the mutant^{1,2} (Fig. 1) may indicate that determination of segments and segment boundaries occurs already at this early stage and hence would not depend entirely on a cell-autonomous expression of *prd*.

The pattern of seven *prd* bands exists only transiently and develops during late syncytial blastoderm into a regular pattern of 13 bands of equal width and spacing. This transition of the pattern exhibiting a two-segment periodicity into one of a single-segment repeat occurs again in an anterior to posterior direction

and spreads from the ventral towards the dorsal side. Such polarities of pattern development have been observed also for *engrailed* (*en*) and *ftz* transcripts²³ and may suggest a common control mechanism. It is interesting that the pair-rule gene *prd*, initially transcribed with a two-segment repeat, is later switched to be expressed with a single-segment periodicity. Spatial expression patterns of *en* (refs 23, 25) and *ftz* (ref. 23) are also switched from longer to shorter periodicities. A switch in the pattern of *ftz* expression occurs also at a later developmental stage, as shown by the finding that the *ftz* gene product is detected in neuroblasts and ganglion cells of every segment^{26,27}. Such changes in patterns would be expected if regulatory interactions among segmentation genes occur and we anticipate that, as more of these genes are studied, more examples of such temporal changes in pattern will be discovered.

The patterns of *en* (refs 23, 28, 29) and *prd* transcripts are very similar at the end of cellular blastoderm. The two most anterior bands of both *en* (ref. 23) and *prd* label the anterior and posterior portion of the cephalic furrow. The two patterns may even coincide as *en* is probably expressed in posterior compartments of each segment^{23,28–31} and our spatial assignments of the *prd* bands are based on those of the *ftz* bands, which may be off by one cell. Thus, although *prd* and *en* are initially under different controls, as apparent from their distribution of transcripts, such a spatial and temporal coincidence of the expression of these two genes during cellular blastoderm might indicate that their control becomes at least partly coordinated at this stage.

Two additional groups of cells begin to express the *prd* gene during late syncytial blastoderm. First, transcripts appear in a dorsal band of cells close to the anterior pole at a location similar to that of *h* expression¹⁰. As has been argued previously¹⁰, these cells may represent the primordium for the labrum³². It remains to be shown, however, whether all of these cells express both *h* and *prd*. Second, a wide posterior band of *prd* transcripts that appears in the posterior region of A8 (and possibly in the primordia for A9, A10 and A11) migrates to the proctodeal invagination during germ-band extension. This band, together with the anterior dorsal band, are the last cells to exhibit *prd* expression during embryogenesis.

We thank Maya Burri for technical assistance, Christiane Nüsslein-Volhard for the *prd* mutant strains and discussions, Michael Levine for the *ftz* clone p523B, Hermann Steller for the cos-P-neo vector and Hans Noll for comments on the manuscript. This work was supported by Swiss NSF grants 3.180-0.82, 3.600-0.84 and 3.600-1.84 and by the Canton of Basel.

Received 1 October 1985; accepted 2 April 1986.

- Nüsslein-Volhard, C. & Wieschaus, E. *Nature* **287**, 795–801 (1980).
- Nüsslein-Volhard, C., Wieschaus, E. & Jürgens, G. *Verh. dt. zool. Ges.*, 91–104 (1982).
- Wieschaus, E. & Gehring, W. *Dev. Biol.* **50**, 249–263 (1976).
- Steiner, E. *Wilhelm Roux Arch. dev. Biol.* **180**, 31–46 (1976).
- Lawrence, P. A. & Morata, G. *Dev. Biol.* **56**, 40–51 (1977).
- Lohs-Schardin, M., Cremer, C. & Nüsslein-Volhard, C. *Dev. Biol.* **73**, 239–255 (1979).
- Szabad, J., Schüpbach, T. & Wieschaus, E. *Dev. Biol.* **73**, 256–271 (1979).
- Hafen, E., Kuroiwa, A. & Gehring, W. J. *Cell* **37**, 833–841 (1984).
- Holmgren, R. *EMBO J.* **3**, 569–573 (1984).
- Ingham, P. W., Howard, K. R. & Ish-Horowitz, D. *Nature* **318**, 439–445 (1985).
- Frei, E., Baumgartner, S., Edström, J.-E. & Noll, M. *EMBO J.* **4**, 979–987 (1985).
- Frei, E. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **50**, 127–134 (1986).
- Scalenghe, F., Turco, E., Edström, J.-E., Pirrotta, V. & Melli, M. *Chromosoma* **82**, 205–216 (1981).
- Nüsslein-Volhard, C., Wieschaus, E. & Kluding, H. *Wilhelm Roux Arch. dev. Biol.* **193**, 267–282 (1984).
- Nüsslein-Volhard, C., Kluding, H. & Jürgens, G. *Cold Spring Harb. Symp. quant. Biol.* **50**, 145–154 (1986).
- Langer-Safer, P. R., Levine, M. & Ward, D. C. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4381–4385 (1982).
- Foe, V. E. & Alberts, B. M. *J. Cell Sci.* **61**, 31–70 (1983).
- Underwood, E. M., Turner, F. R. & Mahowald, A. P. *Dev. Biol.* **74**, 286–301 (1980).
- Sonnenblick, B. P. in *Biology of Drosophila* (ed. Demerec, M.) 62–167 (Wiley, New York, 1950).

- Poulsen, D. F. in *Biology of Drosophila* (ed. Demerec, M.) 168–274 (Wiley, New York, 1950).
- Turner, F. R. & Mahowald, A. P. *Dev. Biol.* **57**, 403–416 (1977).
- Martinez-Arias, A. & Lawrence, P. A. *Nature* **313**, 639–642 (1985).
- Weir, M. P. & Kornberg, T. *Nature* **318**, 433–439 (1985).
- Akam, M. E. & Martinez-Arias, A. *EMBO J.* **4**, 1689–1700 (1985).
- DiNardo, S., Kuner, J. M., Theis, J. & O'Farrell, P. H. *Cell* **43**, 59–69 (1985).
- Carroll, S. B. & Scott, M. P. *Cell* **43**, 47–57 (1985).
- Hiromi, Y., Kuroiwa, A. & Gehring, W. J. *Cell* **43**, 603–613 (1985).
- Kornberg, T., Sidén, I., O'Farrell, P. & Simon, M. *Cell* **40**, 45–53 (1985).
- Fjose, A., McGinnis, W. J. & Gehring, W. J. *Nature* **313**, 284–289 (1985).
- Kornberg, T. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1095–1099 (1981).
- Kornberg, T. *Dev. Biol.* **86**, 363–372 (1981).
- Jürgens, G., Lehmann, R., Schardin, M. & Nüsslein-Volhard, C. *Wilhelm Roux Arch. dev. Biol.* (in the press).
- Southern, E. J. *molec. Biol.* **98**, 503–517 (1975).
- Maniatis, T. *et al.* *Cell* **15**, 687–701 (1978).
- Steller, H. & Pirrotta, V. *EMBO J.* **4**, 167–171 (1985).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: a Laboratory Manual* (Cold Spring Harbor Laboratory Press, New York, 1982).
- Smith, G. E. & Summers, M. D. *Analyt. Biochem.* **109**, 123–129 (1980).
- Laskey, R. A. & Mills, A. D. *FEBS Lett.* **82**, 314–316 (1977).
- Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. *Biochemistry* **16**, 4743–4751 (1977).
- Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
- Hafen, E., Levine, M., Garber, R. L. & Gehring, W. J. *EMBO J.* **2**, 617–623 (1983).
- McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A. & Gehring, W. J. *Nature* **308**, 428–433 (1984).
- Kuroiwa, A., Hafen, E. & Gehring, W. J. *Cell* **37**, 825–831 (1984).

Latitude and depth variation of solar rotation

T. L. Duvall Jr*, J. W. Harvey† & M. A. Pomerantz‡

* Laboratory for Astronomy & Solar Physics, NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

† National Solar Observatory, National Optical Astronomy Observatories, Tucson, Arizona 85726, USA

‡ Bartol Research Foundation, University of Delaware, Newark, Delaware 19711, USA

Measurements of the frequencies of various modes of trapped acoustic waves provide information about rotation and structure within the Sun. Previous work dealt with observations of wave modes confined near the solar equator, which provided some information about the depth variation of rotation without resolving a possible latitude variation^{1,2}. Recent work extended measurements to modes covering various latitude ranges from which the variation with latitude of solar rotation can be studied³⁻⁵. Since these measurements were restricted to modes with spherical harmonic degrees less than 50, they provide averages of rotation over great depth ranges that do not resolve the convective envelope. We now present new results for degrees up to 98 which allow the convective envelope to be isolated. For degrees between 20 and 98 we find no evidence that internal rotation differs significantly with depth or latitude from the rotation of surface magnetic field patterns. Modes covering a wide latitude range have systematically lower frequencies than those confined near the equator, indicating the existence of a structural asymmetry within the Sun.

Modal patterns of trapped acoustic waves are described by the number of nodes of the wave pattern in the radial direction, n , the indices of the spherical harmonic function that characterizes the lateral wave pattern, l (degree) and m (azimuthal order) and cyclic oscillation frequency, ν . Slow rotation causes the frequency to depend on m approximately as⁴

$$\nu(n, l, m) - \nu(n, l, 0) = -m \frac{\int_{-1}^1 \bar{\Omega}(\theta) [P_l^m(\cos \theta)]^2 d \cos \theta}{\int_{-1}^1 [P_l^m(\cos \theta)]^2 d \cos \theta} \quad (1)$$

where θ is the colatitude, $\bar{\Omega}(\theta)$ is an appropriate average of the rotation frequency over the depth range sampled by the particular mode and P_l^m is the associated Legendre function. One may represent a general frequency variation with m as a series

$$\nu(n, l, m) - \bar{\nu}(n, l) = L \sum_{i=0}^{i=N} a_i P_i(-m/L) \quad (2)$$

where $\bar{\nu}$ is the frequency averaged over m , P_i are the Legendre polynomials of degree i and $L = \sqrt{l(l+1)}$. The argument m/L minimizes the innate l dependence of the coefficients. Unlike power series expansions in m used previously, equation (2) produces coefficients that are independent of each other and of the value of N . Expressed in terms of equation (2), the surface differential rotation measured by the motion of magnetic features⁶ (or spectroscopically⁷) yields even-indexed coefficients equal to zero and odd-indexed coefficients (nHz sidereal) $a_1 = 442.8$ (435), $a_3 = 21.7$ (21) and $a_5 = -2.5$ (-4) with negligible l dependences for $l > 20$.

We observed from the geographic South Pole in December 1981 and January 1982, in an effort to obtain sequences of sufficient length to resolve the oscillation spectrum freed from spurious features caused by diurnal interruptions. For the present investigation we used a set of observations started on 24 December 1981 having a duration of 50 h with one 4-h interruption. These data are the best 1/3 of the total collected. The instrument and observing programme have been discussed elsewhere^{8,9}. Briefly, we recorded full-disk intensity images in a spectral band (± 0.6 nm) centred on the Ca II line at 393.3 nm.

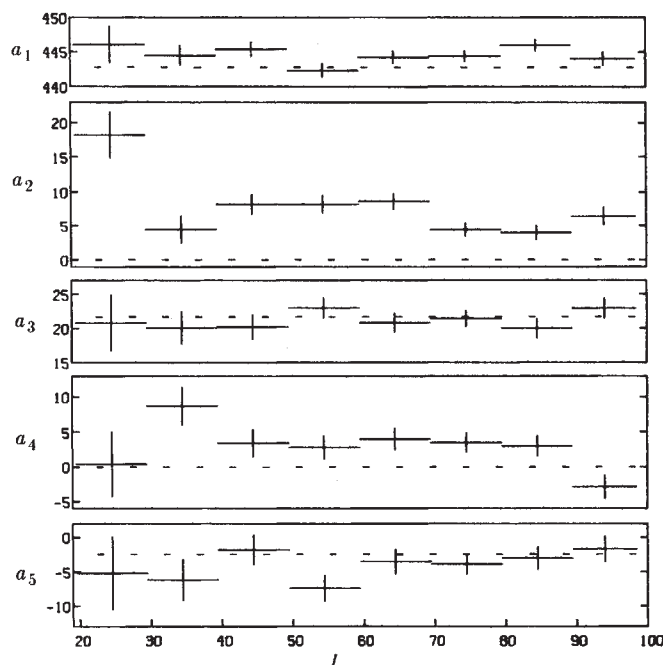


Fig. 1 Averaged coefficients from equation (2) and 1σ random errors as a function of l (nHz). The dashed lines are computed from measurements of the surface differential rotation based on observations of magnetic fields⁶.

This spectral region shows intensity oscillations due to trapped acoustic modes which are several times stronger than adjacent continuum regions^{10,11}. An advantage of intensity observations over the usual Doppler shift observations is a lack of signal weakening near the limb due to projection effects. A disadvantage of intensity observations is greater noise from solar and terrestrial sources¹². Exposures were made with a telescope and a charge-injection-device camera which provided 226 pixels along the solar equator and 178 pixels along the solar rotation axis. Integrations were digitally recorded on magnetic tape cartridges every 90 seconds. After correction of the images for various characteristics of the camera, each image of the visible hemisphere was interpolated onto a longitude, sine latitude grid consisting of 512 by 421 elements. In the manner described by Brown^{3,4}, a Fourier transform in longitude was followed by a transformation by associated Legendre functions in sine latitude and then a Fourier transform in time to produce a spectrum of the solar oscillations with independent variables $l = 0(1)250$, $m = 0(2) \pm l$ and $\nu = 0(5.43)5556 \mu\text{Hz}$. To save computation time and because higher resolution is not justified unless the entire surface of the Sun can be observed at once, the spectrum was computed only for even values of m .

The spectra show tens of thousands of modes. It has not yet been possible to assign the frequency of each mode in order to evaluate equation (2). Instead we used a cross-correlation procedure in a manner similar to Brown^{3,4}. For each l , trial assumptions of $a_1 = 450$ nHz and $a_3 = 0$ were used for modes between

Table 1 Average rotation coefficients and standard deviations (nHz)

l range	a_1	a_2	a_3	a_4	a_5
20-29	446.1 $\pm$ 2.7	18.2 $\pm$ 3.4	20.8 $\pm$ 4.1	0.4 $\pm$ 4.7	-5.2 $\pm$ 5.3
30-39	444.5 $\pm$ 1.5	4.5 $\pm$ 2.0	20.1 $\pm$ 2.4	8.7 $\pm$ 2.7	-6.2 $\pm$ 3.0
40-49	445.4 $\pm$ 1.1	8.2 $\pm$ 1.5	20.2 $\pm$ 1.8	3.4 $\pm$ 2.0	-1.8 $\pm$ 2.2
50-59	442.3 $\pm$ 1.0	8.2 $\pm$ 1.3	23.0 $\pm$ 1.5	2.8 $\pm$ 1.7	-7.4 $\pm$ 1.9
60-69	444.2 $\pm$ 0.9	8.6 $\pm$ 1.2	20.8 $\pm$ 1.4	4.0 $\pm$ 1.6	-3.5 $\pm$ 1.8
70-79	444.4 $\pm$ 0.8	4.4 $\pm$ 1.0	21.4 $\pm$ 1.2	3.5 $\pm$ 1.4	-3.9 $\pm$ 1.5
80-89	446.0 $\pm$ 0.9	4.0 $\pm$ 1.1	20.0 $\pm$ 1.4	3.0 $\pm$ 1.5	-3.0 $\pm$ 1.7
90-98	444.0 $\pm$ 1.0	6.4 $\pm$ 1.3	22.9 $\pm$ 1.5	-2.9 $\pm$ 1.7	-1.7 $\pm$ 1.9

2.4 and 4 mHz to produce a frequency spectrum averaged over m that estimated the spectrum for a non-rotating Sun. This estimate was cross-correlated with the spectrum for each value of m . The frequencies of the peaks of the cross-correlations were then used to improve estimates of $a_{1,3}$. The improved mean spectrum was then cross-correlated with each m -value spectrum to provide values for the solution of equation (2) with $N = 5$. Results for $l < 20$, $l > 98$ were too noisy to be useful. The remaining results were averaged in groups of ten l values and are tabulated in Table 1. The uncertainties in the means are derived from the individual l uncertainties by normal error propagation rules. Individual uncertainties were based on the assumption that the frequency estimation error is independent of m at a given l . The values of a_0 were all less than 1 nHz and are not included in Table 1.

The results are shown in Fig. 1 together with values derived from the measured rotation of surface magnetic field patterns⁶. The odd-indexed coefficients carry information about rotation. There appears to be no significant variation of the rotation coefficients with changing l value. The slight difference between magnetic rotation coefficients and our results is within the range of variation of surface rotation during the solar activity cycle. Thus one may conclude from our results that the rotation averaged over the outer half of the Sun, probed by modes with $l > 20$, is essentially identical with that of the surface. This conclusion requires verification by a formal inversion to deduce the range of possible rotation variations with latitude and depth admitted by the data. Pending such an inversion, we conjecture that differential rotation with latitude persists at least through most of the convection zone.

The even-indexed coefficients provide information about the latitude variation of sound speed versus depth. If the internal structure of the Sun were independent of latitude the even coefficients should be zero. Centrifugal distortion of the Sun would produce a negative value of a_2 (ref. 13). There appears to be a significant, positive difference from zero in the sense that apart from rotation, near-zonal modes have lower frequencies than near-sectoral modes of the same degree. Brown^{3,4} also found a systematic, positive quadratic frequency dependence with m but attributed no significance to it. Transformed to the same basis, his values are about 1/3 of our values. We believe that the effect is genuine and indicates that the structure of the convection zone is different near the equator compared with higher latitudes.

The present measurements may be used to compute the frequency difference to be expected from prograde and retrograde sectoral modes. These calculated frequency differences agree with direct observations of sectoral mode frequencies¹. Brown's⁴ calculated sectoral mode frequency differences also agree with the present results. Unfortunately our results for the latitude dependence of rotation significantly disagree with those of Brown^{3,4}. His values of a_3 for $l = 20-29$, $30-39$, $40-49$ are 4.6 ± 1.2 , 9.1 ± 1.4 and 4.1 ± 2.7 respectively, or roughly 1/3 of our values. Brown's rotation results indicate much less differential rotation with latitude than we find.

We have examined many possible sources of this discrepancy without identifying a satisfactory explanation. Some possibilities which have been examined and are now considered unlikely are: (1) simple numerical error, (2) different frequency ranges used in the analyses, (3) the different spatial responses of intensity and velocity, (4) the cross-correlation technique responding to (in the intensity observations) the solar background signal rotating at the surface rate and (5) the Sun changing with time. Some more likely possibilities which have been considered and which require additional evaluation include: (1) the different frequency resolution, (2) the different temporal sidelobe structure and (3) the surface pattern of intensity modulating the oscillations and changing the spatial response functions. Further observations should reveal the source of the systematic error. Although Libbrecht's observa-

tions⁵ do not cover the degree range that we observed, his measurements substantially agree with those of Brown^{3,4} at l values less than 20.

This work was done while T.L.D. was a visiting astronomer at the National Solar Observatory in Tucson. R. Aikens of Photometrics, Ltd and R. Pfeiffer of Bartol greatly assisted the success of our observations from the South Pole. R. Nagel of NOAO designed and assembled the data recording system. We appreciate discussions with T. Brown aimed at resolving the discrepancy between our separate results. P. Goode pointed out an error in the original version of equation (2). This work was supported in part by NSF grant DPP78-22267. The National Optical Astronomy Observatories are operated by the Association of Universities for Research in Astronomy, Inc. under contract with NSF.

Received 18 February; accepted 26 March 1986.

1. Duvall, T. L. Jr & Harvey, J. W. *Nature* **310**, 19-22 (1984).
2. Duvall, T. L. Jr *et al.* *Nature* **310**, 22-25 (1984).
3. Brown, T. M. *Nature* **317**, 591-594 (1985).
4. Brown, T. M. in *Seismology of the Sun and the Distant Stars* (ed. Gough, D. O.) 199-214 (Reidel, Dordrecht, 1986).
5. Libbrecht, K. G. *Nature* **319**, 753-755 (1986).
6. Snodgrass, H. B. *Astrophys. J.* **270**, 288-299 (1983).
7. Snodgrass, H. B. *Solar Phys.* **94**, 13-31 (1985).
8. Harvey, J., Pomerantz, M. & Duvall, T. Jr *Sky Telesc.* **64**, 520-523 (1982).
9. Pomerantz, M. A., Harvey, J. W. & Duvall, T. Jr *Antarctic J.U.S.* **17**(5), 232-233 (1982).
10. Jensen, E. & Orrall, F. Q. *Astrophys. J.* **138**, 252-270 (1963).
11. Duvall, T., Livingston, W. & Mahaffey, C. in *Nonradial and Nonlinear Stellar Pulsation* (eds Hill, H. A. & Dziembowski, W. A.) 237-240 (Springer, Berlin, 1980).
12. Harvey, J. in *Future Missions in Solar Heliospheric & Space Plasma Physics* (eds Rolfe, E. & Battick, B.) 199-208 (ESA, Noordwijk, 1985).
13. Gough, D. O. & Taylor, P. P. *Mem. Soc. Astron. Ital.* **55**, 215-226 (1984).

Superparamagnetic component in the Orgueil meteorite and Mössbauer spectroscopy studies in applied magnetic fields

M. B. Madsen*, S. Mørup*, T. V. V. Costa†‡, J. M. Knudsen† & M. Olsen†

* Laboratory of Applied Physics II, Technical University of Denmark, DK-2800 Lyngby, Denmark

† Physics Laboratory I, H. C. Ørsted Institute, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark

The Orgueil meteorite is a carbonaceous chondrite of group CI. Wdowiak and Agresti¹ have recently shown that very small magnetic particles, which exhibit superparamagnetic relaxation below 80 K, are present in this meteorite. They suggested that these small particles consist of magnetite. Using Mössbauer spectroscopy in external magnetic fields we have found that the Orgueil meteorite does not contain superparamagnetic magnetite in any appreciable amount. The meteorite contains another, as yet unidentified iron (III) compound which exhibits superparamagnetic behaviour below 80 K.

The sample of the carbonaceous chondrite Orgueil was ground into a powder and a Mössbauer absorber was prepared in a plexiglass absorber holder. Mössbauer spectra were obtained at various applied magnetic fields and temperatures.

Figure 1 shows a series of Mössbauer spectra of the meteorite recorded at various temperatures without applied magnetic fields. The spectra obtained at 298 and 125 K show the presence of magnetically split components due to iron in the tetrahedral (A) and octahedral (B) sites of magnetite². The area ratio of

‡ Permanent address: Departamento de Física, UFC POBox 3004, 60000 Fortaleza, Brazil.

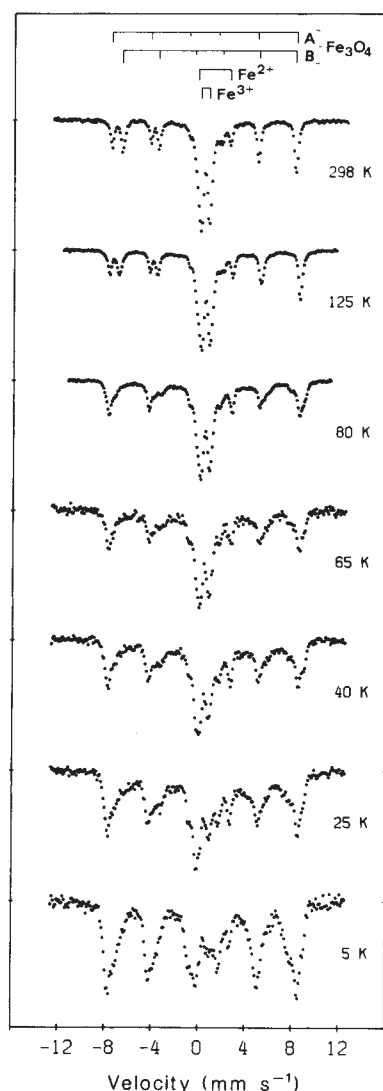


Fig. 1 Mössbauer spectra of the Orgueil meteorite obtained at different temperatures. The bar diagram indicates the tetrahedral (A) and octahedral (B) sites of the magnetite and the Fe^{2+} and Fe^{3+} doublets.

the A and B components indicates that the magnetite is slightly more oxidized than ideal stoichiometric magnetite, in which this area ratio is approximately 1:2. The spectra also contain quadrupole doublets due to paramagnetic Fe^{2+} and Fe^{3+} . At room temperature the Fe^{3+} doublet has a quadrupole splitting $\Delta E_Q = 0.73 \pm 0.03 \text{ mm s}^{-1}$ and an isomer shift relative to $\alpha\text{-Fe}$, $\delta_{\text{Fe}} = 0.37 \pm 0.02 \text{ mm s}^{-1}$. The line width is $\Gamma = 0.46 \pm 0.02 \text{ mm s}^{-1}$. For the Fe^{2+} component we find $\Delta E_Q = 2.75 \pm 0.08 \text{ mm s}^{-1}$, $\delta_{\text{Fe}} = 1.13 \pm 0.04 \text{ mm s}^{-1}$, $\Gamma = 0.33 \pm 0.02 \text{ mm s}^{-1}$. The Mössbauer parameters of the Fe^{2+} components are in accordance with Fe^{2+} ions in a hydrated silicate^{3,4}.

Below the Verwey transition temperature ($\sim 119 \text{ K}$)⁵ the Mössbauer spectrum of magnetite is very complex and as many as six sextets may be required to fit the spectrum adequately⁶. The spectra of Fig. 1 reveal that the relative area of the iron (II) doublet remains nearly constant, at least down to 25 K. However, below 65 K, the relative area of the iron (III) doublet gradually decreases with decreasing temperature, whereas the total area of the magnetically split components increases. The observed behaviour is typical for a system containing superparamagnetic microcrystals with a distribution of particle sizes^{7,8}. Thus the spectra indicate that Fe^{3+} ions are present in a compound which exhibits superparamagnetic relaxation in this temperature range. On the basis of similar spectra Wdowiak and

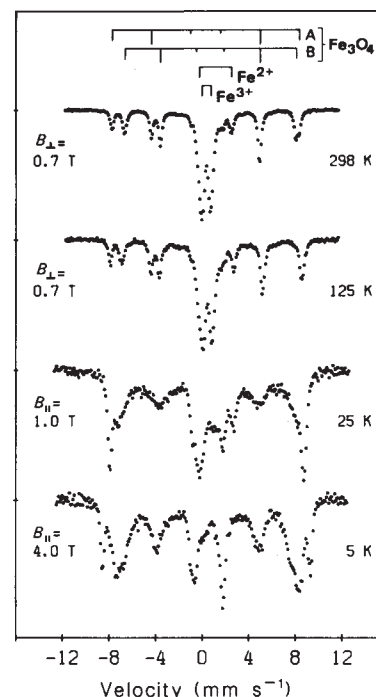


Fig. 2 Mössbauer spectra of the Orgueil meteorite obtained at different temperatures with the absorber in external magnetic fields. The intensity and direction of the external field, B , with respect to the γ -rays is indicated.

Agresti¹ concluded that the Orgueil meteorite contains superparamagnetic magnetite particles.

Because of the overlap of many different magnetically split components in the low-temperature spectra, it is difficult to determine the exact Mössbauer parameters of the six-line component which appears at low temperature at the expense of the Fe^{3+} doublet. Mössbauer studies in applied magnetic fields may, however, give supplementary information on superparamagnetic microcrystals and, in particular, such studies can give information on the magnetic moments of the microcrystals^{7,8}.

Therefore, to clarify whether the mineral that exhibits superparamagnetic relaxation above 5 K and contributes the Fe^{3+} doublet above 80 K has any net magnetic moment, Mössbauer spectra were obtained with external magnetic fields applied to the sample. The spectra are shown in Fig. 2. At 298 and 125 K a magnetic field of 0.7 T was applied perpendicular to the γ -ray direction. This leads to a change in the area ratios of the magnetite sextet lines from $\sim 3:2:1:1:2:3$ to $3:4:1:1:4:3$, indicating alignment of the magnetization direction parallel to the applied field. The doublets are only slightly affected by the magnetic fields; that is, the width of their lines increases.

Comparing the parameters with those of the spectrum obtained in zero field, it was found that the relative areas of the doublets and the sextets do not change significantly on application of the external field. Previous studies^{7,8} of samples of superparamagnetic magnetite particles with average particle diameters down to 4 nm have shown a substantial splitting of the spectra when similar magnetic fields were applied. This is due to the fact that the Zeeman energy is comparable with, or larger than, the thermal energy even at room temperature and, therefore, the particles are significantly magnetized by the external field. The present results, therefore, show that the quadrupole doublet is not due to superparamagnetic magnetite particles, as was suggested by Wdowiak and Agresti.

Further information about the nature of the superparamagnetic phase can be obtained from the spectra measured at 25 and 5 K with applied magnetic fields parallel to the γ -ray direction, shown in Fig. 2. From these spectra it is seen that the

magnetization direction of the magnetite aligns parallel to the applied magnetic field. This alignment leads to the disappearance of the $\Delta m = 0$ lines, that is, the lines 2 and 5 of the magnetite sextets. Some broad lines remain, however, at velocities where the magnetite should not give absorption lines. The broad lines at about -4 and $+5 \text{ mm s}^{-1}$ thus indicate the presence of an iron-containing compound with no significant net magnetization. This is the compound that gives rise to the iron(III) doublet at higher temperatures. The magnetic hyperfine fields of this compound are about 47 T at 25 K and 49 T at 5 K. The large line widths ($>1 \text{ mm s}^{-1}$) of this compound suggest that it is a poorly crystallized or perhaps amorphous iron (III) compound. The low net magnetization of the compound could be due to antiferromagnetic ordering.

From our results we conclude that the superparamagnetic component in the Orgueil meteorite is not due to magnetite particles. The meteorite contains particles of another iron (III) compound that has a negligible net magnetization, but exhibits a superparamagnetic behaviour below 65 K.

The carbonaceous chondrites of group CI have an elemental composition closely resembling that of the Sun, if we disregard the most volatile elements. These meteorites are believed to have condensed in the very early phase of the formation of the Solar System. Even if the carbonaceous chondrites are partially altered, for example by aqueous activity, their chemical composition may still have some similarity to interstellar, and circumstellar, dust⁹. If this is so, the findings of Wdowiak and Agresti¹ and the results of the present work indicate that superparamagnetism is a much more common phenomenon in the Universe than hitherto believed.

The sample of the meteorite was supplied by the Mineralogical Museum of Copenhagen. The project is supported by the Danish Natural Science Research Council. T.V.V.C. is supported by the Brazilian Agency CAPES.

Received 23 December 1985; accepted 7 March 1986.

1. Wdowiak, T. J. & Agresti, D. G. *Nature* **311**, 140–142 (1984).
2. Häggström, L. *et al. Hyperfine Interactions* **5**, 201–214 (1978).
3. Nagy, B. *Carbonaceous Meteorites* (Elsevier, Amsterdam 1975).
4. Coey, J. M. D. in *Mössbauer Spectroscopy Applied to Inorganic Chemistry* (ed Long, G. J.) 443–509 (Plenum, New York, 1984).
5. Verwey, E. J. W. & Haayman, P. W. *Physica* **8**, 979–987 (1941).
6. Rubinstein, M. & Forester, D. W. *Solid State Commun.* **9**, 1675–1679 (1971).
7. Mørup, S., Dumesic, J. A. & Topsøe, H. in *Applications of Mössbauer Spectroscopy*, Vol. II, 1–53 (ed. Cohen, R. L.) (Academic, New York, 1980).
8. Mørup, S., Topsøe, H. & Clausen, B. S. *Phys. Scr.* **25**, 713–719 (1982).
9. Cameron, A. G. W. & Pine, M. R. *Icarus* **18**, 377–406 (1973).

Gaseous ammonia and ammonium ions in the free troposphere

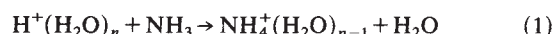
H. Ziereis & F. Arnold

Max-Planck-Institut für Kernphysik, Postfach 103980,
D-6900 Heidelberg, FRG

Ammonia, the most important alkaline compound commonly found in the atmosphere, has a key role in aerosol chemistry. Gaseous ammonia is released at the Earth's surface primarily through the decomposition of organic material and, due to its extremely large solubility in water and reactivity with acid aerosol components, is efficiently removed by interaction with aqueous and acid aerosols. While the presence of ammonium ions (NH_4^+) in aerosols and rain water is well established, there have been few studies of gaseous ammonia and its abundance is not well established, particularly in the free troposphere¹. This is probably due to the difficulty in developing sufficiently sensitive analytical methods, which are not affected by interference of ammonium-containing aerosols. Due

to its large proton affinity or gas-phase basicity, ammonia vapour is expected to react also with atmospheric gaseous positive ions leading to gaseous NH_4^+ 'core ions'. Detection of the gaseous NH_4^+ core ions, which have not yet been observed in the free atmosphere, may provide sensitive means of detecting atmospheric ammonia vapour^{2,3,4}. We report here on the first measurements of this ion in the free atmosphere at altitudes from 4,000 to 8,000 m. Ammonia vapour abundances inferred from these data are very low suggesting efficient heterogeneous ammonia removal and very low ammonia saturation pressures over aerosols.

Due to its large proton affinity ($204.0 \text{ kcal mol}^{-1}$), ammonia vapour reacts with most atmospheric positive ion species². This is particularly true for $\text{H}^+(\text{H}_2\text{O})_n$ clusters, which are quickly formed from primary positive ions created in the troposphere by cosmic rays and radioactivity and react with ammonia vapour:



(rate coefficient: $k = 1.8 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ for $n = 3$; (ref. 5)).

Also $\text{H}^+\text{CH}_3\text{CN}(\text{H}_2\text{O})_n$ clusters, which were recently found in the middle and lower stratosphere⁶, undergo reactions analogous to (1) and the rate coefficients, as recently measured in our laboratory⁷, are $1.8 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ ($n = 0$), $1.8 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ ($n = 1$) and $1.2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ ($n = 2$). Reactions (1) involving proton hydrate ions with n around 8 are also fast, having rate coefficients around $1 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ as we have recently found by creating large $\text{H}^+(\text{H}_2\text{O})_n$ clusters in ambient air and letting them react with ambient ammonia vapour. Using this active chemical ionization (ACIMS) method and taking the above $k = 1.8 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$, we obtained a ground-level ammonia vapour mixing ratio of 3.2 p.p.b.v. (parts per 10^9 by volume) which falls well inside the range of previous ammonia measurements employing denuder, chemical filter, and condensation collection techniques (0.5–10 p.p.b.v.; see refs 8, 9). Thus, it seems fairly well established that NH_4^+ -containing clusters should be found in the Earth's atmosphere provided that ammonia vapour is sufficiently abundant.

We have used an improved aircraft-borne ion mass spectrometer with high mass resolution and sensitivity to search for NH_4^+ -containing cluster ions. This instrument was similar to one used previously^{10–14} and so will not be described here. It has three modifications: (1) A larger quadrupole mass spectrometer (rod diameter: 1.6 cm) providing higher mass resolution and sensitivity; (2) a larger cryopump (liquid neon-cooled) adding to the improved sensitivity; (3) an ECA (electric field-induced collisional activation) mode used for fragment ion studies facilitating ion identification and adding to an improved overall sensitivity.

Three flights of the modified ion mass spectrometer were carried out aboard a twin-jet research aircraft of the DFVLR (Deutsche Forschungs- und Versuchsanstalt für Luft- und Raumfahrt). The flights (F11, F12, F13), which took place on 11, 14, and 15 May 1985 over the FRG, covered a total altitude range from ~4,000 to 10,000 m.

When the ECA mode was used, the major positive ion mass peaks occurred within the mass range 12–25 AMU (atomic mass units). Therefore, this portion of the mass spectrum was preferably scanned to increase the effective sampling time and thereby increase the sensitivity for ion detection which is particularly helpful at high mass resolution. Figure 1a, b, and c show mass spectra as obtained in flight F12 at different altitudes. These mass spectra contain three major peaks at 19, 18, and 15 AMU which are due to H_3O^+ , NH_4^+ and CH_3^+ . To ensure that the two peaks of 18 and 19 AMU are due to two different ion species and not an artefact resulting from a bad peak shape (peak splitting), an in-flight calibration was carried out. Here, $\text{H}^+(\text{H}_2\text{O})_n$ clusters were created using the ACIMS mode of the instrument employing a high-frequency glow discharge ion source¹³.

A typical ACIMS spectrum as shown in Fig. 1d contains

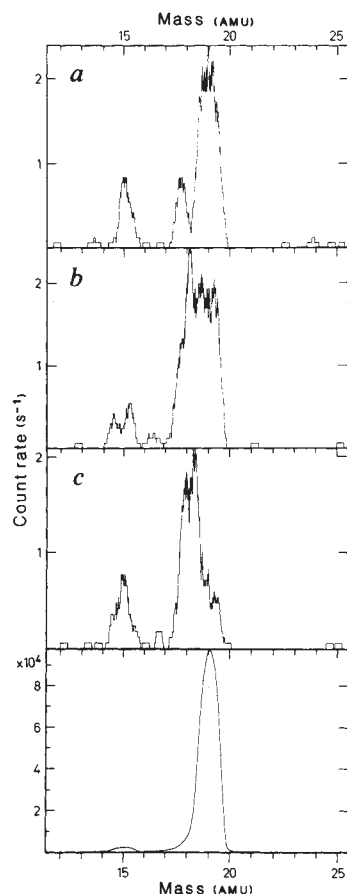


Fig. 1 Portions of positive ion mass spectra as obtained on flight F12 at altitudes of: *a*, 6,900 m; *b*, 6,000 m; *c*, 5,100 m. For comparison an ACIMS spectrum is also shown (*d*), obtained on the same flight at 8,000 m and serving as an in-flight calibration of the mass scale and the peak shape.

mainly a peak at mass 19 (H_3O^+). The peak shape of 19 is nominal with no peak splitting present. While H_3O^+ represents a core of ambient cluster ions, CH_3^+ is probably a fragment of core ions made up by a protonated molecule containing a methyl group as, for example, $\text{H}^+\text{CH}_3\text{CN}$ (protonated acetonitrile), $\text{H}^+(\text{CH}_3)_2\text{CO}$ (protonated acetone) or $\text{H}^+(\text{C}_3\text{H}_6)$ (protonated propylene). The spectra shown in Fig. 1*a-c* also reveal a marked change of the abundance ratio for NH_4^+ and H_3O^+ taking place around 6,000 m. Figure 2*a* shows the height variation of this ion abundance ratio. It increases from 0.1 (lower limit of measurements) at 10,000 m to 10 (upper limit of measurements) at 4,000 m. At 6,000 m where most data were obtained, a marked variability was also found even during a single flight (F11). The time span between the two extreme F11 measurements at 6,000 m was only about 15 min corresponding to a horizontal distance of about 200 km. Weather conditions were characterized by clouds reaching up to about 3,000 m (F13) and no clouds present (F11, F12). Local tropopause levels were 9,800 m (F11), 11,570 m (F12), and 9,130 m (F13).

The ammonia vapour abundance can be roughly estimated from our ion composition data by assuming that NH_4^+ -containing ions are formed from the other observed ions (mainly H_3O^+ -containing ions) and lost by ion-ion recombination (typical¹⁵ tropospheric ion-ion recombination lifetime: $t_R = 100$ s). A steady-state treatment yields the following continuity equation:

$$(\text{H}_3\text{O}^+)k(\text{NH}_3) = (\text{NH}_4^+)t_R^{-1} \quad (2)$$

(brackets denote number densities). H_3O^+ and NH_4^+ stand for

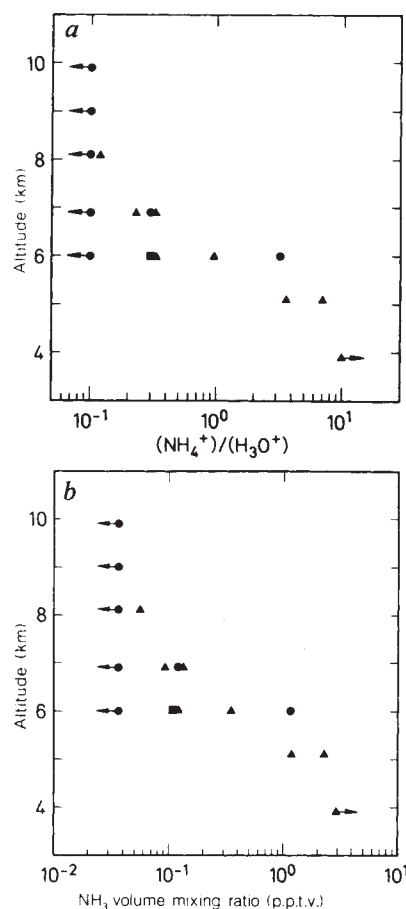
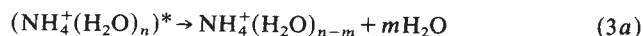


Fig. 2 *a*, Abundance ratios for NH_4^+ and H_3O^+ 'core' ions as measured in the aircraft flights given in the insert. Arrows denote upper or lower limits. *b*, Ammonia vapour volume mixing ratios as inferred from the data given in *a*. ●, F11, 11 May 1985; ▲, F12, 14 May 1985; ■, F13, 15 May 1985.

clusters containing these 'cores'. Applying this passive chemical ionization mass spectrometry (PACIMS) method and taking $k = 2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$ (see above) and $t_R = 100$ s, one obtains ammonia vapour abundances as given in Fig. 2*b* ranging between ~ 0.043 and 3.5 p.p.t.v. (parts per 10^{12} by volume). The uncertainties for k , t_R , and the measured $(\text{NH}_4^+)/(\text{H}_3\text{O}^+)$ ratio are about $\pm 50\%$, $\pm 50\%$, and $\pm 30\%$, respectively. Thus, the uncertainty for the inferred (NH_3) is about $\pm 80\%$. An additional systematic error may arise if ECA-induced fragmentation of $\text{NH}_4^+(\text{H}_2\text{O})_n$ clusters



(the asterisk denotes that the $\text{NH}_4^+(\text{H}_2\text{O})_n$ cluster is internally highly excited) gives rise not only to H_3O^+ (equation (3a)) but also to NH_4^+ (3b). As can be judged from our above mentioned laboratory ACIMS measurements, process (3b) should be much less efficient than process (3a). However, even if there was only a minor side channel leading to H_3O^+ large errors could be induced for ratios of ambient $\text{NH}_4^+(\text{H}_2\text{O})_n$ and $\text{H}^+(\text{H}_2\text{O})_n$ ions being much larger than one. In such conditions, the PACIMS and ACIMS methods may underestimate ambient ammonia vapour abundances.

Compared with the two upper tropospheric ammonia vapour measurements of Hoell *et al.*¹⁶, who used an infrared heterodyne radiometer technique (the only upper tropospheric ammonia vapour measurements previously reported) and obtained values between about 0.6 and 2 p.p.b.v., our present data are much

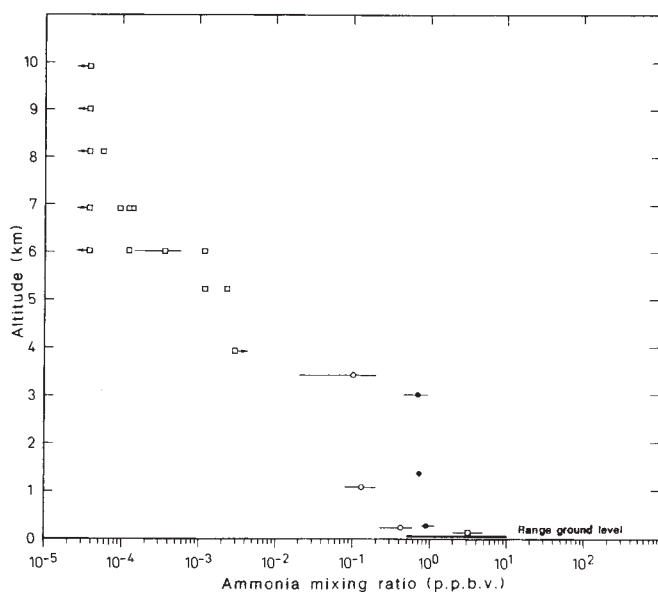


Fig. 3 Comparison of present ammonia vapour data ($\square$) and previous denuder measurements by Lebel *et al.*¹⁷: $\circ$, March 1983; $\bullet$, September, 1983. A typical range for previous ground level measurements at continental sites^{8,9} is also given ($\triangle$). Also shown is a typical ground level value obtained from active chemical ionization mass spectrometry during tests of the present method (see text).

lower. All other ammonia vapour measurements previously reported are restricted to heights below about 3,500 m and, therefore, a direct comparison with the present data is not possible. Recent aircraft-borne denuder measurements by Lebel *et al.*¹⁷ (Fig. 3), extending up to 3,500-m range between about 0.1 and 1 p.p.b.v. Ground-level values, as mentioned before, typically fall into the range between about 0.5 and 10 p.p.b.v. (Fig. 3) being mostly much larger than free tropospheric values.

The observed decrease with height of the ammonia vapour volume mixing ratio suggests that an efficient sink for this gas must be operative throughout the troposphere. This sink can probably be identified as ammonia vapour interaction with aerosols of various sorts ultimately followed by washout and rainout of the aerosols. Even in fair weather conditions, the surface area densities of continental background aerosols are sufficiently large to make the lifetime t_c of a gaseous ammonia molecule against collision with aerosols much smaller than typical timescales t_{VT} for tropospheric vertical transport. Consequently, a very steep decrease with height of the ammonia vapour volume mixing ratio should be expected if ammonia reevaporation was negligible. On average, the aerosol content decreases with increasing height and therefore t_c increases. Considering typical aerosol abundances¹ and sizes, one estimates tropospheric t_c values to be much less than typical timescales for tropospheric vertical transport ($\sim 2 \times 10^4$ – 2×10^6 s km⁻¹). Thus, in many conditions, a local equilibrium between ammonia absorption by and re-evaporation from aerosols may be established depending sensitively on the aerosol composition. In reality, ambient aerosols are complex systems whose chemical and physical states are governed by atmospheric conditions and often vary with time and location. Depending on the extent of neutralization and atmospheric relative humidity, they may exist in a completely dissolved, partially dissolved or crystalline state as pure or mixed salts. For example, solid NH_4NO_3 , a relatively simple aerosol, would have ammonia vapour equilibrium saturation volume mixing ratios¹⁹ (ammonia equilibrium saturation pressures converted to volume mixing ratios) of about 0.37 p.p.b.v. (4,000 m), 0.15 p.p.b.v. (5,200 m), 0.06 p.p.b.v. (6,100 m), 0.03 p.p.b.v. (7,000 m), and 0.003 p.p.b.v.

(8,200 m) if atmospheric temperatures as measured in F12 are considered. Compared with our measured ammonia abundances, these are generally larger by factors around 10–100. For NH_4HSO_4 and $(\text{NH}_4)_2\text{SO}_4$ aerosols the equilibrium saturation abundance for ammonia vapour may be much smaller than for NH_4NO_3 depending sensitively on the molar ratio $\text{NH}_4^+/\text{SO}_4^{2-}$ and relative humidity²⁰. At ground level (temperature of 25 °C and a relative humidity of 85%) for example, the equilibrium ammonia vapour pressure over ammonium sulphates (molar ratio $\text{NH}_4^+/\text{SO}_4^{2-} = 1$) is about 50 times lower than over NH_4NO_3 .

We conclude that it is at least conceivable that our measured ammonia vapour abundances represent equilibrium abundances over ambient aerosols. If so, they may contain valuable information on the chemical nature of aerosols. Future measurements should focus on variations of ambient ammonia vapour abundances with atmospheric temperature which should be very pronounced if ammonia reevaporation was in fact important. An attempt should be made to carry out combined measurements of ammonia vapour and other relevant parameters including H_2SO_4 , HNO_3 , and H_2O vapours as well as atmospheric temperatures.

We thank E. E. Ferguson and C. Brosset for helpful discussions, and the technical staffs of the Max-Planck-Institut für Kernphysik, particularly K. Oberfrank and W. Dann and of the Deutsche Forschungs- und Versuchsanstalt für Luft- und Raumfahrt (Flugbereitschaft), for their support. Part of this project was funded by the Bundesministerium für Forschung und Technologie through Gesellschaft für Strahlen- und Umweltforschung.

Received 13 November 1985; accepted 10 March 1986.

- Graedel, T. E. & Weschler, C. J. *Rev. Geophys. Space Sci.* **19**, 505–539 (1981).
- Ferguson, E. E. & Fehsenfeld, F. C. *J. chem. Phys.* **59**, 6212 (1973); *Gas phase Chemistry* (ed. Bowers, M. T.) (Academic, New York, 1979).
- Arnold, F. *Proc. Dahlem Workshop on Atmospheric Chemistry* (ed. Goldberg, E. D.) 273 (1982).
- Arnold, F. in *Chemical Events in the Atmosphere and their Impact on the Environment* (Pontificiae Academiae Scientiarum Scripta Varia, 1985).
- Bohme, D. K., Mackay, G. I. & Tanner, S. D. *J. Am. chem. Soc.* **101**, 3724 (1979).
- Arnold, F., Böhlinger, H. & Henschen, G. *Geophys. Res. Lett.* **5**, 653–xxx (1978).
- Schlager, H., Fabian, R. & Arnold, F. *Proc. 3rd Swarm Seminar, Innsbruck*, 257–262 (1983).
- Dawson, G. A. & Farmer, J. C. *J. geophys. Res.* **89**, 4779–4787 (1984).
- Ferm, M. *Atmos. Envir.* **13**, 1385–1393 (1979).
- Heitmann, H. & Arnold, F. *Nature* **306**, 747 (1983).
- Arnold, F., Heitmann, H. & Oberfrank, K. *Planet. Space Sci.* **12**, 1567 (1984).
- Hauck, G. & Arnold, F. *Nature* **311**, 547 (1984).
- Arnold, F. & Hauck, G. *Nature* **315**, 307 (1985).
- Knop, G. & Arnold, F. *Planet. Space Sci.* (submitted).
- Arnold, F., Knop, G. & Ziereis, H. *Nature* (in the press).
- Hoell, J. M., Harward, C. N. & Williams, B. S. *Geophys. Res. Lett.* **7**, 313–316 (1980).
- Lebel, P. J., Hoell, J. M., Levine, J. S. & Vay, A. *Geophys. Res. Lett.* **12**, 401–404 (1985).
- Liu, S. C. & McAfee, J. R. *J. geophys. Res.* **89**, 7291–7297 (1984).
- Brandner, J. D., Junk, N. M., Lawrence, J. W. & Robina, J. J. *Chem. Engng Data* **7**, 227–228 (1962).
- Tang, I. N. *Atmos. Envir.* **14**, 819–828 (1980).

Acetone measurements in the upper troposphere and lower stratosphere—implications for hydroxyl radical abundances

F. Arnold, G. Knop & H. Ziereis

Max-Planck-Institut für Kernphysik, Postfach 103 980,
D-6900 Heidelberg, FRG

Atmospheric acetone serves as a precursor of PAN (peroxyacetyl nitrate) which represents a temporary reservoir for reactive nitrogen and has a potential to form aerosols. Acetone has recently been detected in the atmosphere by Penkett², using ground-level flask sampling and gas chromatography/mass spectrometry (GC/MS) analysis in the laboratory finding an average volume mixing ratio of 470 p.p.t.v. (parts per 10¹² by volume) in Atlantic

air. Very recently, acetone was for the first time detected in the free atmosphere, at heights around the tropopause, by Arnold and his colleagues^{3,4} using aircraft-borne chemical ionization mass spectrometry (CIMS). We report here on the first measurements of atmospheric acetone abundances over an extended altitude range (5,900–11,300 m) covering the lowermost part of the stratosphere and the upper troposphere. The measured upper tropospheric abundances (average 120 p.p.t.v.) are substantially lower than the average values (470 p.p.t.v.) measured at ground level. Above the tropopause, a marked decrease of the acetone mixing ratio was observed. Our present data have interesting implications for the chemistry of non-methane hydrocarbons and possibly also hydroxyl radical abundances.

Aircraft-borne CIMS, used for the present measurements, is similar to the experimental method previously employed by our group^{3–7} and will only briefly be reviewed here. It involves a flow reactor through which atmospheric air is passed and within which ions created in the air stream by a high-frequency glow discharge ion source undergo ion molecule reactions with atmospheric trace gases. Precursor and product ions are then mass analysed using a cryogenically-pumped quadrupole mass spectrometer. Abundances of reactant trace gases can be determined from measured ion abundance ratios and independently measured ion residence times.

The present CIMS-instrument has several modifications to the previous model including a larger quadrupole mass spectrometer (quadrupole rod diameter: 1.6 cm) and an ECA (electric field-induced collisional activation) mode. The larger quadrupole provides a larger sensitivity and mass resolution while the ECA mode is used for fragmented ion mass spectrometry studies, which greatly facilitates ion identification. A detailed description of the modified instrument will be given elsewhere.

Six flights (F6, F8, F9, F11, F12, F13) were carried out over the FRG using a twin-jet research aircraft (type: FALCON 20) of the Deutsche Forschungs- und Versuchsanstalt für Luft- und Raumfahrt. The flights, which all took place during daytime, covered a total altitude range from 5,900 to 11,300 m.

The acetone volume mixing ratios measured during these flights are shown in Fig. 1a along with the single data point of our first CIMS-flight (F5 (ref. 4)). Some of the data were obtained at altitudes above the local tropopause levels. Figure 1b shows the same data, but replotted as a function of the vertical separation from the local tropopause level. These data exhibit substantial scatter, but a trend also seems to exist towards lower values above the tropopause which is particularly pronounced for F6 and F8 where altitude profiles were obtained covering both the uppermost part of the troposphere and the lowermost part of the stratosphere. Our stratospheric data (obtained at heights at least 500 m above local tropopause levels) range between ~20 and 50 p.p.t.v. (average: 31 p.p.t.v.) whereas our tropospheric data range between ~50 and 250 p.p.t.v. (average: 120 p.p.t.v.).

Compared with the ground-level average of 470 p.p.t.v. our upper tropospheric average of 120 p.p.t.v. is substantially lower which, if both values were representative, would imply that efficient tropospheric acetone sinks must exist. Known sinks include photolysis and OH-attack of which the former process appears to be dominant. The photodissociation frequency J is about $1\text{--}2 \times 10^{-6} \text{ s}^{-1}$ at the 10-km level⁸ which implies an acetone lifetime of about 10 to 20 days (diurnal average). The reaction coefficient¹ for OH-attack is $2 \times 10^{-13} \text{ cm}^3 \text{ s}^{-1}$ which implies a typical lifetime of about 53 days (diurnal average) in summer at middle latitudes (here a typical diurnal average OH-concentration of $1 \times 10^6 \text{ cm}^{-3}$ as given by the model of Crutzen⁹ was taken). By comparison, the timescale for vertical mixing¹⁰ between ground level and the 10 km level is about 10–100 days mostly exceeding the photochemical lifetime of acetone. Consequently, substantial upward mixing of acetone originating from the planetary boundary layer has to be expected. Taking a lower limit to the photochemical lifetime of acetone of 10

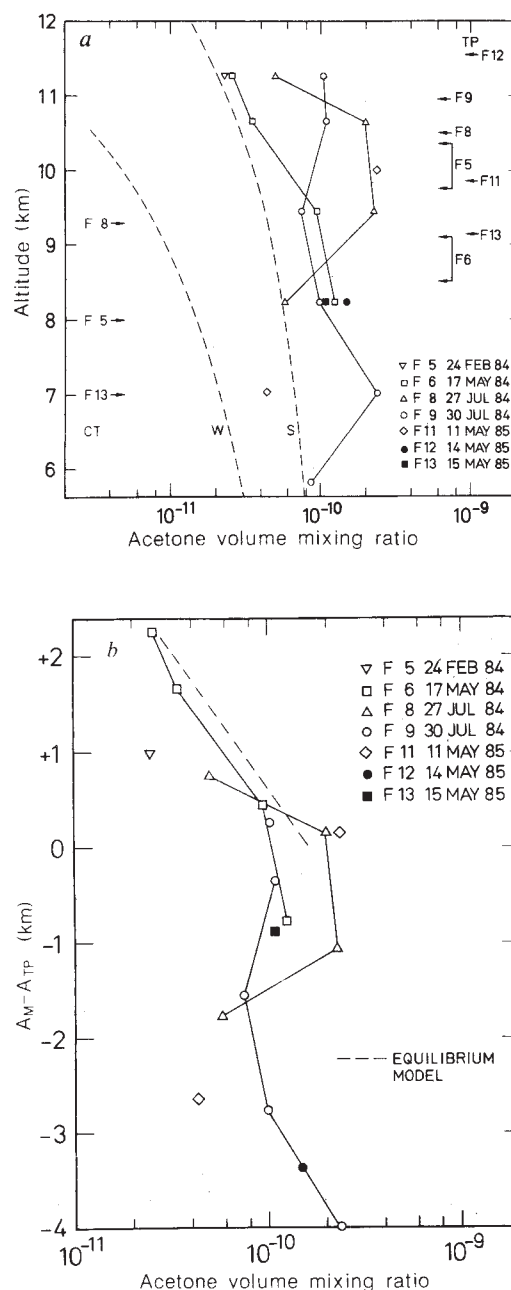


Fig. 1 a, Acetone volume mixing ratios as measured during the aircraft flights (F6, F8, F9, F11, F12, F13; see inset) along with the single data point of our previous flight (F5; ref. 4). Also given are local tropopause (TP) and cloud top (CT) levels. Model curves for a gas with a surface source, a 10-day lifetime and a ground-level mixing ratio of 470 p.p.t.v. are also shown for winter (W) and summer (S) conditions. b, As a with vertical separation from local tropopause level (A_M is the altitude of the measurement and A_{TP} the local tropopause level) instead of geometric altitude as the y-coordinate. A model curve assuming local photochemical equilibrium in the stratosphere between acetone formation by propane photooxidation and loss by photolysis is also shown (dashed curve).

days, assuming a ground-level mixing ratio of 470 p.p.t.v., and neglecting photochemical acetone production above the ground, average winter and summer volume mixing ratio profiles as shown in Fig. 1 would be expected. Here, recent vertical eddy diffusion coefficients as given by Liu *et al.*¹⁰ were considered. Compared with our data (Fig. 1a), the model curves are gen-

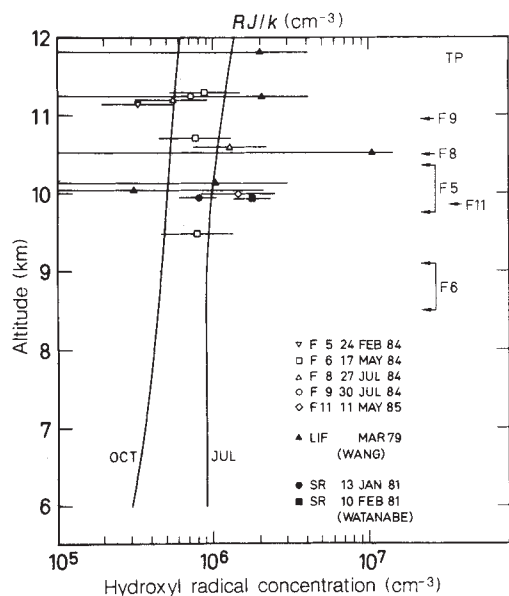
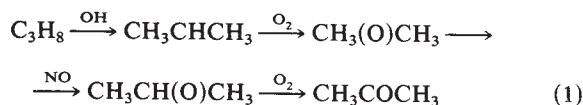


Fig. 2 Inferred stratospheric RJ/k values (see text), which would be equal to OH-concentrations (diurnal averages) for local photochemical equilibrium of acetone formation by $C_3H_8 + OH$ and acetone formation by $C_3H_8 + OH$ and acetone destruction by photolysis. For comparison, previous OH-measurements using laser-induced resonance fluorescence¹⁵ (Wang) and spin trapping¹⁶ (Watanabe), and the photochemical model calculations (diurnal averages for July and October at mid-altitudes) of Crutzen⁹ are shown.

erally lower, particularly for the only winter data point (F5). This discrepancy may be due to a longer photochemical lifetime or faster vertical transport. The latter may occur during strong convective events of deep penetration like a mid-latitude storm when vertical transport may eventually occur within about 1–3 days¹¹. However, it seems unlikely that all of our measurements were influenced by such events. An additional potential cause for the observed discrepancy is local photochemical production of acetone by photooxidation of hydrocarbons. It has been proposed by Singh and Hanst¹ that acetone may be formed by photooxidation of propane through the reaction chain:



which is initiated by the OH-radical and also involves nitric oxide as a reactant. Propane is emitted at ground level mainly as a result of natural gas losses and biomass burning¹² and reaches the upper troposphere and even the lower stratosphere since it has a photochemical lifetime of about 6–12 days which is mainly determined by OH-attack. Propane measurements^{12,13} give free tropospheric volume mixing ratios at middle latitudes of about 50–1,000 p.p.t.v. (most values ranging between 100 and 300 p.p.t.v.) and rapidly decreasing values above the tropopause. At ground level¹³, propane volume mixing ratios range between about 600–1,000 p.p.t.v. at middle latitudes being substantially higher than the upper tropospheric average of about 120 p.p.t.v.. It is conceivable that photooxidation of other, possibly even more reactive atmospheric hydrocarbons may contribute to the formation of acetone. In the stratosphere, the situation is different. Here vertical mixing becomes much slower having a typical timescale¹⁰ of 100–200 days km^{-1} which is much longer than the photochemical lifetime of acetone. Therefore, the

acetone concentration may be determined by local photochemical production and destruction by photolysis. However, here only relatively long-lived hydrocarbons like propane serve as potential precursors. Considering only photooxidation of propane one obtains the following continuity equation:

$$[C_3H_8]k[OH] = (CH_3)_2CO J^{-1} \quad (2)$$

Taking stratospheric propane volume mixing ratios as measured by Rudolph and Ehhalt¹², an OH-reaction coefficient k of $1.82 \times 10^{-11} \exp(-675/T) \text{ cm}^3 \text{ s}^{-1}$ as measured by Darnall and co-workers¹⁴, and OH concentrations for summer conditions at middle latitudes as given by the theoretical model of Crutzen⁹ (see Fig. 2, curve Jul), one obtains a photochemical equilibrium volume mixing ratio profile as shown in Fig. 1b. It is in reasonably good agreement with our stratospheric data. However, this agreement may be fortuitous since substantial temporal and spatial variations of lower stratospheric propane abundances may occur. Combined measurements of acetone and propane at the same time and location are required to investigate the local photochemical acetone production.

Finally, we consider the potential role of acetone as a tracer for hydroxyl radicals. In conditions where local photochemical equilibrium is established, which may be the case in the lower stratosphere, combined measurements of acetone and propane may allow to estimate the OH-concentration by applying the continuity equation (2). To illustrate the kind of information, which may be obtained, we have inferred the quantity RJ/k ($R = ((CH_3)_2CO)/(C_3H_8)$), which should be equal to the OH-concentration (diurnal average) for local photochemical equilibrium conditions, from our data using propane volume mixing ratios as above (same as those taken for calculating the theoretical curve in Fig. 2). Resulting stratospheric RJ/k values are given in Fig. 3 along with published OH-measurements previously made in the tropopause region using laser-induced fluorescence¹⁵ (only measurements with no O_3 -interference are included) and spin trapping¹⁶. While our data represent diurnal averages, the above previous data were obtained during daytime, mostly at large solar elevations. As OH should be absent during the nighttime, diurnal average values should be smaller than daytime values by about a factor of two to three. Considering this fact, our inferred values are roughly consistent with previous data. Theoretical model calculations (diurnal averages) by Crutzen⁹ are also shown in Fig. 2.

We thank E. E. Ferguson, D. Ehhalt, P. Crutzen, and R. Chatfield for helpful discussions. Support by the technical staffs of the Max-Planck-Institut für Kernphysik, in particular K. Oberfrank and W. Dann, and of the Deutsche Forschungs- und Versuchsanstalt für Luft- und Raumfahrt (Flugbereitschaft) is gratefully appreciated. Part of this project was funded by the Bundesministerium für Forschung und Technologie through Gesellschaft für Strahlen- und Umweltforschung.

Received 18 November 1985; accepted 11 March 1986.

- Singh, H. B. & Hanst, P. L. *Geophys. Res. Lett.* **8**, 941–944 (1981).
- Penkett, S. A. in *Atmospheric Chemistry* (ed. Goldberg, E. D.) (Springer, Berlin, 1982).
- Hauck, G. & Arnold, F. *Nature* **311**, 547–550 (1984).
- Arnold, F. & Hauck, G. *Nature* **315**, 307–309 (1985).
- Heitmann, H. & Arnold, F. *Nature* **306**, 747–751 (1983).
- Arnold, F., Heitmann, H. & Oberfrank, K. *Planet. Space Sci.* **32**, 1567–1576 (1984).
- Knop, G. & Arnold, F. *Planet. Space Sci.* **33**, 983–986 (1985).
- Gardner, E. P., Ranmali, D., Wijayarathne, R. D. & Calver, J. G. *J. phys. Chem.* **88**, 5069 (1984).
- Crutzen, P. in *Atmospheric Chemistry* (ed. Goldberg, E. D.) 313–328 (Springer, Berlin 1982).
- Liu, S. C., McAfee, J. R. & Cicerone, R. C. *J. geophys. Res.* **89**, 7291 (1984).
- Chatfield, R. B. & Crutzen, P. J. *Geophys. Res.* **89**, 7111 (1984).
- Ehhalt, D. H. & Rudolph, J. *KFA Rep.* ISSN 0366-0885 (July 1984).
- Rudolph, J. & Ehhalt, D. H. *J. geophys. Res.* **86**, 11959 (1981).
- Darnall, K. R., Atkinson, R. & Pitts, J. N. Jr *J. Phys.* **82**, 1581 (1978).
- Wang, C. C., Davis L. I. Jr & Selzer, P. M. *J. geophys. Res.* **86**, 1181–1186 (1981).
- Watanabe, T. et al. *Analyst. Chem.* **54**, 2470–2474 (1982).

Precipitation scavenging of aerosol particles over remote marine regions

Patrick Buat-Ménard* & Robert A. Duce†

* Centre des Faibles Radioactivités, Laboratoire Mixte CNRS-CEA, BP 1, 91190 Gif-sur-Yvette, France

† Center for Atmospheric Chemistry Studies, Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881, USA

There is increasing evidence that the deposition of atmospheric particulate material has a significant influence on the chemistry of trace elements in many oceanic regions¹⁻⁴. One significant but unknown parameter is the efficiency of in-cloud and below-cloud scavenging processes as a function of aerosol particle size. Studies in continental regions have indicated that the largest particles are scavenged most efficiently by rain⁵. We report here, for the first time, an evaluation of particle scavenging by tropical showers, based on data obtained at Eniwetok Atoll and American Samoa in the tropical Pacific Ocean. We find no clear evidence of a particle size dependence for scavenging in this remote marine region. This indicates that estimates of global atmospheric deposition over the ocean based on extrapolation of measurements made on land may be invalid.

Because of the regional variability of both the atmospheric and oceanic cycles of some trace elements, especially trace metals, an assessment of the impact on ocean chemistry of the deposition of atmospheric particulate material from natural or man-made sources must go beyond simple estimates of global mass balances. The need for accurate data on net air-to-sea particulate fluxes requires a precise assessment of the importance of both dry and wet deposition processes. Such data are extremely difficult to obtain, especially over remote marine regions.

Wet deposition appears to be the dominant removal process for particulate material from the remote marine atmosphere^{3,6}; there is thus a need to understand how the concentrations of trace elements in rain are related to their concentrations in the atmosphere. The investigation of the relationship between particle size and scavenging efficiency over remote marine areas is of crucial importance, because the various sources contributing to the marine atmospheric burden of particulate trace elements generate aerosol particles with different mass-size functions.

The data reported here were obtained during the SEAREX Program experiments in 1979 at Eniwetok Atoll (11°20' N, 162°20' E) in the Marshall Islands, and in 1981 at American Samoa (14°15' S, 170°34' W). Both sites are >5,000 km from the nearest continent. Trade-wind rain-shower and atmospheric particulate samples were collected from the top of an 18-m-high tower on the windward shore of the islands. Samples were collected only during sustained on-shore winds, using ultra-clean procedures. Rain was collected on an event basis, whereas aerosol particle samples were generally collected over 2-5 days. Two different data sets are presented here which allow us to investigate the precipitation scavenging efficiency for aerosol particles as a function of particle size.

The first data set^{4,7} consists of results of chemical analyses of 14 pairs of rain and air samples for trace elements at Eniwetok Atoll. These samples were analysed by neutron activation analysis and atomic absorption spectrometry. The air samples were obtained with high-volume cascade impactors, which separate aerosol particles into six different size ranges. Because the elements analysed are partitioned differently among particles of different size⁷, we can examine the dependence of element concentrations in rain and air on aerosol particle size. Elements have been characterized by the mass median diameter (MMD)

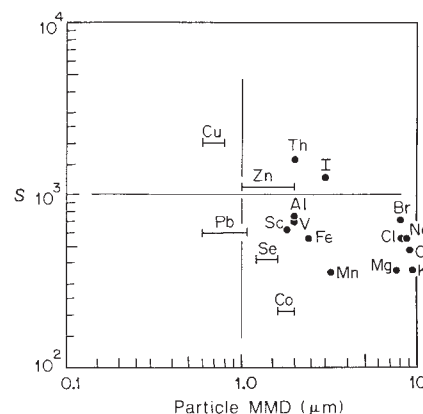


Fig. 1 Scavenging ratio, S , plotted against marine airborne particle size (mass median diameter) for various trace elements at Eniwetok Atoll.

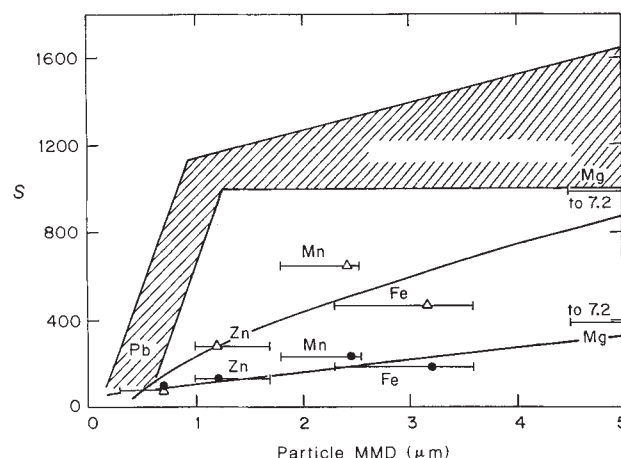


Fig. 2 Scavenging ratio, S , plotted against particle size for various trace elements in urban areas (adapted from ref. 5). The shaded area indicates the relationship observed in Chilton, UK¹⁰; the solid lines are for two sites near St Louis, USA⁹.

of the particles containing them. Although the aerosol samples were collected over intervals of several days, whereas the rain samples represent aerosol scavenging on a timescale of minutes to hours, the aerosol trace-metal size distributions were found to be quite consistent over a period of several months at Eniwetok. Comparison of rain and aerosol chemistry is thus justified.

The second data set⁸ was obtained by means of scanning-electron-microscopic examinations of individual aluminosilicate dust particles in air and rain samples at Eniwetok and American Samoa. Such particles are nearly insoluble in rain water; therefore, these data should provide a direct estimate of wet removal efficiencies as a function of particle size.

The precipitation scavenging efficiency for a given substance is commonly expressed as a dimensionless number, the scavenging ratio (or washout factor) S , given by $S = C_{\text{rain}} \rho / C_{\text{air}}$, where C_{rain} is the concentration in rain in g kg^{-1} , C_{air} is the concentration in air in g m^{-3} and ρ is the density of air (1.2 kg m^{-3}) at standard conditions.

In Fig. 1, the mean scavenging ratios for elements in rain samples from trade-wind showers at Eniwetok Atoll are plotted against the mass median diameter of the aerosol particles containing them. There is no statistically significant correlation between scavenging ratio and particle size ($r = -0.39$, not significant at $P = 0.1$). This result is corroborated by the data

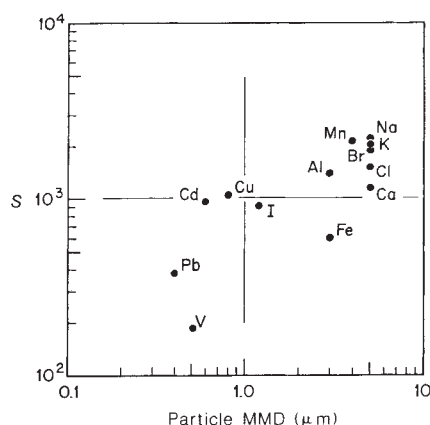


Fig. 3 Scavenging ratio, S , plotted against particle size for various trace elements in the Florida Keys (adapted from ref. 11).

Table 1 Scavenging ratios for mineral aerosols, Eniwetok and American Samoa

Particle diameter (μm)	S
0.1–0.5	$940 \pm 1,100$
0.5–1	800 ± 620
1.0–1.5	500*
1.5–3	310 ± 250
3.0–6	270 ± 170
6–15	320 ± 120

Scavenging ratio S is given as arithmetic mean $\pm 1\sigma$, based on five air samples and three rain samples. Details of the particle counting statistics can be found in ref. 8. 100–400 particles were examined from each sample. The size distributions were found to be log-normal. Scavenging ratios were then calculated from the idealized log-normal distributions of the particles in air and rain samples. The stated uncertainties relate only to the variability of the particle size distribution from one sample to another.

* Assumed value (see text).

presented in Table 1 for aluminosilicate particles collected at Eniwetok and American Samoa, which show no statistical difference (at the 1σ level) among the values of S for the different size fractions. The data presented in this table indicate only the relative variation in S , because the data have been normalized to $S = 500$ for aerosol particles in the size range 1–1.5 μm .

If what we have observed is representative of the trade-wind regime, it can be inferred that over tropical marine areas the elemental concentration ratios in near-surface air and rain may be similar. This conclusion may also apply to the westerlies regime of the South Pacific, as indicated by recent analyses of individual aluminosilicate particles in air and rain samples collected during the SEAREX Program experiment in 1983 at Ninety-Mile Beach, New Zealand (P.B.-M., unpublished data).

The results presented here, which are to our knowledge the first of this type obtained over remote marine regions, contrast markedly with data obtained over continental and coastal areas. For example, Scott⁵ has shown that S increases with MMD for metals collected in St Louis⁹, USA, and Chilton, UK¹⁰ (Fig. 2), and Duce *et al.*¹¹ have found similar results in the Florida Keys (Fig. 3) ($r = 0.76$, significant at $P = 0.01$). These results may be explained by the lower collection efficiency of raindrops for the smaller particles (down to perhaps a few tenths of a micrometre in radius) present over the continents^{5,12}. These authors also note that differing chemical properties, particularly the solubil-

ity, of particles of different size may also play a role. Over remote marine regions, the absence of a particle size dependence of scavenging ratios is as yet unexplained. Slinn¹² has suggested that in truly remote marine regions, where there are relatively few cloud condensation nuclei ($< 200 \text{ cm}^{-3}$), essentially all particles are activated in clouds regardless of their size or chemical composition. This suggestion deserves further investigation, but there are other possible causes which might explain the results reported here.

Indeed, it can be argued that such a continental-marine comparison should result in differences, because for a given element the vertical distribution of its atmospheric concentration may be quite different over land from that over the sea. For example, elements (such as sodium) associated with atmospheric sea-salt particles exhibit a marked negative concentration gradient between the sea surface and the top of the marine boundary layer^{13,14}, whereas such a gradient is much less pronounced or non-existent over land. The reverse is generally observed for materials attached to aerosol particles over the ocean which are derived from land-surface sources^{14,15}. Such differences in vertical distribution may generate large uncertainties in the calculation of S , because S values are estimated from near-surface air concentrations, which can be quite different from those at the altitude of cloud scavenging. However, such uncertainties should affect S values similarly for materials during their atmospheric transport from land to sea or from sea to land. That this is not observed is clear from Figs 1–3, which show that for elements derived from continental sources the shift of S values from continental or coastal regions to remote oceanic areas is not uniform for a given element, and seems to be related to the mass-size distribution of the element. Indeed, this shift is much more pronounced for elements (such as aluminium) attached to large aerosol particles than for those (such as lead) attached to smaller aerosol particles.

Another possible cause for the continental-marine difference is related to precipitation patterns over land and sea. Differences in the vertical and horizontal extent of cloud systems and in the duration, intensity and droplet size of the precipitation will certainly affect the precipitation scavenging efficiency of aerosol particles and the relationship between S and the mass-size distribution of the materials associated with these particles. As shown by Scott⁵, large variations in S can be expected at different latitudes. For example, smaller scavenging ratios would be expected in 'cold clouds', where a much greater fraction of the precipitation occurs as a result of vapour-phase growth rather than droplet coalescence. The latter apparently dominates in 'warm' rains, that is, those not containing ice particles or super-cooled droplets. This obviously implies that our limited data set may not apply to high-latitude oceanic areas.

The data presented here suggest strongly that the particle-size-dependent rain scavenging relationships observed in continental regions do not apply in tropical marine regions, where precipitation is often dominated by trade-wind showers. These apparent differences in scavenging processes must be evaluated more fully before accurate models can be made of the wet deposition of aerosol trace constituents into the ocean. Such modelling will become increasingly necessary in response to the growing interest in the use of new oceanic tracers^{16,17}, especially those (such as heavy metals) which are derived from aeolian inputs of anthropogenic origin.

We thank R. Arimoto and G. Ayers for helpful discussion and criticism. This research was supported by the NSF Division of Ocean and Atmospheric Sciences under grants OCE77-13071, OCE77-13072, OCE81-11895 and OCE 84-05605. This is CFR contribution no. 724.

Received 18 November 1985; accepted 7 January 1986.

1. Cambray, R. S., Jeffries, D. F. & Topping, G. *U.K. atom. Energy author. Publ. no. AERE-R7733* (HMSO, London, 1975).
2. Buat-Menard, P. & Chesselet, R. *Earth planet. Sci. Lett.* **42**, 399–411 (1979).

3. Settle, D. M. & Patterson, C. C. *J. geophys. Res.* **87**, 8857-8869 (1982).
4. Arimoto, R., Duce, R. A., Ray, B. J. & Unni, C. K. *J. geophys. Res.* **90**, 2391-2408 (1984).
5. Scott, B. C. *Atmospheric Pollutants in Natural Waters* (ed. Eisenreich, S. J.) 3-21 (Ann Arbor Science, 1981).
6. Uematsu, M., Duce, R. A. & Prospero, J. M. *J. atmos. Chem.* **3**, 123-138 (1985).
7. Duce, R. A., Arimoto, R., Ray, B. J., Unni, C. K. & Harder, P. J. *J. geophys. Res.* **88**, 5321-5342 (1983).
8. Buat-Menard, P., Ezat, U. & Gaudichet, A. *Precipitation Scavenging, Dry Deposition and Resuspension* Vol. 2, 1259-1270 (Elsevier, New York, 1983).
9. Gatz, D. F. *Precipitation Scavenging—1974* (eds Beadle, R. W. & Semonin, R. G.) 71-87 (U.S. Energy Res. Dev. Ag., Washington, DC, 1975).
10. Cawse, P. A. *U.K. atom. Energy Auth. Publ. no. R7669* (HMSO, London, 1974).
11. Duce, R. A. *et al.* in *Abstr. IAMAP/CACGP Symp. Budget and Cycles of Trace Gases and Aerosols in the Atmosphere* (University of Colorado Press, Boulder, 1979).
12. Slinn, W. G. N. *Air-Sea Exchange of Gases and Particles* (eds Liss, P. S. & Slinn, W. G. N.) 299-405 (Reidel, Dordrecht, 1983).
13. Blanchard, D. C. & Woodcock, A. H. *Ann. N.Y. Acad. Sci.* **338**, 330-347 (1980).
14. Delaney, A. C., Pollack, W. H. & Shedlovsky, J. P. *J. geophys. Res.* **78**, 6249-6268 (1973).
15. Gillette, D. A. & Bli-ford, I. H. *J. atmos. Sci.* **28**, 1199-1210 (1971).
16. Gwinn, E. & Sarmiento, J. L. *A Model for Predicting Strontium-90 Fallout in the Northern Hemisphere* (1954-1974) (Ocean Tracers Lab., Princeton University, 1984).
17. Sarmiento, J. L. & Gwinn, E. *J. geophys. Res.* (in the press).

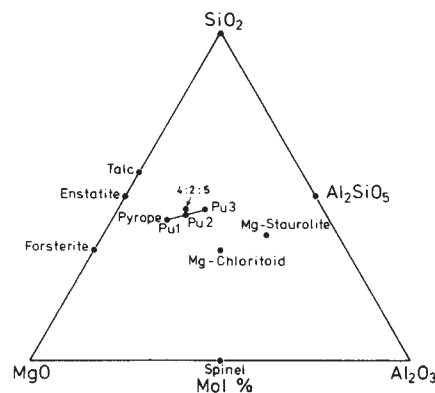


Fig. 1 Projection from H₂O of the MgO-Al₂O₃-SiO₂-H₂O system, showing the possible extent of solid solution of the Ca-free pumpellyite phase (Pu 1 to Pu 3), as well as the bulk composition 4:2:5 of the critical run discussed in the text.

Calcium-free pumpellyite, a new synthetic hydrous Mg-Al-silicate formed at high pressures

W. Schreyer, W. V. Maresch, O. Medenbach & T. Baller

Institut für Mineralogie, Ruhr-Universität Bochum, D-4630 Bochum, FRG

The mineral pumpellyite, $W_4X_2Y_4Z_6O_{(20+t)}(OH)_{(8-t)}$ where $W = \text{Ca, Mn}$; $X = (\text{Mg, Fe}^{2+}, \text{Mn})_{2-t}$; $Y = \text{Fe}^{3+}, \text{Al}$; $Z = \text{Si}$ (ref. 1) is a well-known constituent of very low-grade metamorphic rocks, especially of metabasic compositions. Schiffmann and Liou² have synthesized the ideal Fe-free endmember 'Mg-Al' pumpellyite, $\text{Ca}_4\text{MgAl}_5\text{Si}_6\text{O}_{21}(\text{OH})_7$, and found its upper thermal stability to lie near 375-400 °C in the range of 5-8 kbar water pressure. Here we report the high-pressure synthesis of a pumpellyite-type phase in which the position W of the above formula is solely occupied by magnesium. This 'Mg-Mg-Al pumpellyite', which is chemically nearly equivalent to pyrope + H₂O, crystallizes at 40-55 kbar, 700-850 °C. Therefore, it qualifies as a potential hydrous mantle phase in a subduction zone regime.

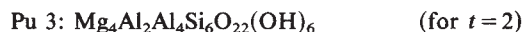
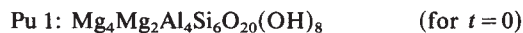
During a recent high-pressure investigation of the system MgO-Al₂O₃-SiO₂-H₂O (MASH) aimed at synthesizing a Ti-free ellenbergerite³, reflections were encountered in the powder X-ray diffraction pattern that did not fit any of the known crystalline phases of the MASH system, nor could they be related to an ellenbergerite variety. In a run with a gel of the composition $4\text{MgO} \cdot 2\text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2$ plus excess water at 700 °C, 50 kbar water pressure, for 5 days, these unknown peaks became dominant, while only small amounts of talc could be identified in addition. A comparison of the unknown peaks with those of potentially related silicate phases suggested a pumpellyite-type crystal structure. This was confirmed by successful indexing of more than 40 observed reflections below 70° 2θ CuKα, on the basis of a monoclinic cell. The refined cell dimensions, using an internal *F*-phlogopite standard, are given in Table 1. Observed diffraction data for the most prominent reflections of the new phase to a *d*-spacing of 2,000 are given in Table 2. Compared with the synthetic Ca-bearing 'Mg-Al pumpellyite' of Schiffmann and Liou², also listed in Table 1, the unit cell volume of the synthetic Ca-free phase is ~9% smaller. Under the microscope, the new phase forms small tabular crystals (4 × 10 × 40 μm) with negative elongation for all orientations. The refractive indices listed in Table 1 are appreciably higher than those of the synthetic Ca-bearing phase.

The crystal structure of pumpellyite^{4,5} has Ca in sevenfold

coordination with respect to oxygen and relatively short Ca—O distances of ~2.43 Å. Following the straightforward rule of increasing coordination number with pressure, it is most likely that, at the high pressure of synthesis, Mg is able to substitute for Ca in the sevenfold positions. Thus a general formula of the synthetic MASH pumpellyite can be expected to be



Since t can vary only between zero and 2, we can define the following theoretical compositions of Ca-free pumpellyites, which are here called 'Mg-Mg-Al pumpellyites':



In Fig. 1, these three compositions are plotted in the MAS compositional triangle and connected through the line of mutual theoretical substitution $\text{Mg} + \text{H} = \text{Al}$. Note that in this projection Pu 1 plots together with pyrope, $\text{Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$. Pu 2 and 3 can be represented, for example, by the three-phase assemblage pyrope + SiO₂ + Al₂SiO₅.

Figure 1 also shows the projection point of the gel composition 4:2:5 used as starting material for the successful synthesis. This composition lies very close to the theoretical 'Mg-Mg-Al pumpellyite' Pu 2. Indeed, the observed products of synthesis, 'Mg-Mg-Al pumpellyite' plus small amounts of talc, seem to indicate that this new pumpellyite phase has a composition close to that of Pu 2 and may even be identical to it. Note that in the system CaO-MgO-Al₂O₃-SiO₂-H₂O, 100% yield of Mg-Al pumpellyite could only be achieved with a bulk composition having $t = 1$ as in Pu 2 (ref. 2).

No reliable information is available as to the thermodynamic pressure/temperature (P/T) stability of the new pumpellyite-type phase. In other MASH synthesis runs at 40 kbar/700 °C, some of the characteristic X-ray powder diffraction lines were discerned as well. In addition, these reflections were observed, together with others, in chemically similar mixtures of the system MgO-BeO-Al₂O₃-SiO₂-H₂O run in the P/T range 45-55 kbar, 700-850 °C (ref. 6). Nevertheless, all these products could be metastable. Seeded runs are required to resolve this problem.

The geoscientific importance of the high-pressure synthesis reported here lies in the fact that 'Mg-Mg-Al pumpellyite' is, as a first approximation, chemically equivalent to pyrope garnet + water. As this garnet is one of the minerals making up the Earth's mantle at depths ≥60-80 km, the question arises as to whether or not this garnet can be replaced in the mantle

Table 1 Physical properties of synthetic Ca-bearing pumpellyite and the new Ca-free pumpellyite phase

Parameter	Ca ₄ MgAl ₅ Si ₆ O ₂₁ (OH) ₇ *	Ca-free phase†
<i>a</i> (Å)	8.825(8)	8.540(1)
<i>b</i> (Å)	5.875(5)	5.712(1)
<i>c</i> (Å)	19.10(1)	18.481(2)
β (°)	97.39(7)	97.71(1)
<i>V</i> (Å ³)	982.1	893.4(1)
<i>n_x</i>		1.674(2)
<i>n_y</i>	Average	1.6825(10)
<i>n_z</i>	1.624	1.699(2)
2 <i>V</i> (°)	—	72(1)
<i>D_x</i> (g cm ⁻³)	3.04	~3.3

* Synthetic; † data from this paper.

Table 2 The most prominent reflections of Ca-free pumpellyite observed for *d* > 2.000

<i>d</i> (Å)	<i>I</i> ^{rel}	<i>hkl</i>	<i>d</i> (Å)	<i>I</i> ^{rel}	<i>hkl</i>
9.18	7	002	2.728	12	022
8.51	3	100	2.651	48	20 $\bar{6}$
4.67	24	11 $\bar{1}$	2.548	58	31 $\bar{1}$
4.58	33	004	2.463	3	311
4.50	22	111	2.423	37	024
4.23	6	200			{ 220
4.05	3	20 $\bar{2}$	2.367	100	{ 11 $\bar{7}$
3.891	3	11 $\bar{3}$	2.252	31	222
3.659	62	202	2.244	28	313
3.410	16	21 $\bar{1}$	2.225	4	30 $\bar{6}$
3.339	21	204	2.208	5	31 $\bar{5}$
2.918	28	204	2.170	11	22 $\bar{4}$
2.856	15	020	2.137	55	20 $\bar{8}$
2.846	41	213	2.125	39	40 $\bar{2}$
		{ 300	2.086	23	026
2.798	84	{ 30 $\bar{2}$	2.041	23	224
		{ 115	2.027	18	404

material by the new pumpellyite phase, provided water is available and the temperatures are sufficiently low for hydration. If 'Mg-Ag-Al pumpellyite' were stable, for example, at 40 kbar and 800 °C, this phase could be a constituent of a mantle plate which is being subducted along a low geothermal gradient of ~7.5 °C km⁻¹ to ~120 km. Such low geothermal gradients and subduction to at least 100 km were recently inferred even for crustal rocks⁷. The water required for the formation of the pumpellyite phase would be readily available in the serpentinized upper portions of an oceanic mantle. This model becomes even more attractive when one considers the possibility of solid solution in the pumpellyite phase, involving both Mg and Ca in the *W*-position of the structure, with concomitant stabilization towards lower water pressures. The new phase is also of interest in relation to a series of hydrous synthetic high-pressure phases in the system MgO-SiO₂-H₂O (refs 8, 9) which have largely unknown structures, and are also discussed as possible receptacles of water in the Earth's mantle.

Received 13 January; accepted 2 April 1986.

1. Coombs, D. S., Nakamura, Y. & Vuagnat, M. *J. Petrol.* **17**, 440-471 (1976).
2. Schiffman, P. & Liou, J. G. *J. Petrol.* **21**, 441-474 (1980).
3. Schreyer, W., Baller, T. & Chopin, C. *Terra cognita* **5**, 327 (1985).
4. Galli, E. & Alberti, A. *Acta crystallogr. B* **25**, 2276-2281 (1969).
5. Yoshiasa, A. & Matsumoto, T. *Am. Miner.* **70**, 1011-1019 (1985).
6. Hölischer, A., Schreyer, W. & Lattard, D. *Contr. Miner. Petrol.* **92**, 113-127 (1986).
7. Chopin, C. *Contr. Miner. Petrol.* **86**, 107-118 (1984).
8. Ringwood, A. & Major, A. *Earth planet. Sci. Lett.* **2**, 130-133 (1967).
9. Yamamoto, K. & Akimoto, S. *Am. J. Sci.* **277**, 288-312 (1977).

A new ³⁶Cl hydrological model and ³⁶Cl systematics in the Jordan River/Dead Sea system

M. Paul*, A. Kaufman†, M. Magaritz†, D. Fink*, W. Henning‡, R. Kaim§, W. Kutschera‡ & O. Meirav*

* Racah Institute of Physics, The Hebrew University, Jerusalem, Israel 91904

† Isotope Department, Weizmann Institute of Science, Rehovot, Israel 76100

‡ Argonne National Laboratory, Argonne, Illinois 60439, USA

§ Nuclear Physics Department, Weizmann Institute of Science, Rehovot, Israel 76100

The recent breakthrough in our ability to detect the radioactive isotope ³⁶Cl (half-life *T*_{1/2} = 301,000 yr) at natural levels by accelerator mass spectrometry¹ allows the processes of salination of water systems to be studied in a new way by distinguishing the chloride content originating in young rainwaters and their subsequent evaporation from that generated by the leaching of ancient rocks. Results for the Jordan River/Dead Sea system show that the amount of chloride leached from rocks ranges from ~70% in source springs to >90% in water bodies downstream. Furthermore, the amount of water left after evaporation decreases from ~50% in the source springs to 20% in the intermediate Lake Kinneret. In the terminal Dead Sea, 99% of the stable chloride originates from ancient rocks and evaporite formations while ~80% of its ³⁶Cl content is of meteoric origin. Using ³⁶Cl measurements, we estimate the accumulation time of the Dead Sea salt to be 19,000–25,000 yr.

Meteoric ³⁶Cl enters the hydrological cycle together with stable chloride from aerosols when they are both washed out of the atmosphere by precipitation. The evaporation and evapotranspiration (through vegetation) of surface and soil water, which follow soon after, are not expected to alter significantly the chlorine isotopic ratio, although they increase the chloride concentration. In contrast, the addition of chloride leached from rocks with a ³⁶Cl/Cl ratio generally much lower than that of precipitation, reduces this isotopic ratio. The chloride concentration is further increased by the evaporation which occurs in rivers and lakes after ground water emerges in springs.

It is extremely difficult to distinguish between the processes of evaporation (including evapotranspiration) and of rock leaching by means of standard hydrological methods, as both processes result in concentration increases. But independent measurement of ³⁶Cl/Cl ratios and of stable chloride concentrations permit the quantitative determination of the extent to which each of the two processes has occurred in a given system.

We denote the chloride concentration of a water body and of precipitation by *C_w* and *C_p*, respectively (in mg l⁻¹), their respective ³⁶Cl/Cl ratios as *R_w* and *R_p*, and the ³⁶Cl/Cl ratio in the rock leachate by *R_L*. The fraction of the total chloride in the water body which originated in the rock leachate is then given by:

$$f_L = (1 - R_w/R_p)/(1 - R_L/R_p) \quad (1)$$

The evaporation factor (EF), defined as the ratio of the volume of the water body to the volume of the precipitation from which it originated, may then be calculated from:

$$EF = (C_p/C_w)/(1 - f_L) \quad (2)$$

These equations demonstrate how the processes of evaporation and rock chloride addition may be distinguished by measuring the quantities *C_p*, *C_w*, *R_p*, *R_w* and *R_L*. If *R_L/R_p* < 1 (as expected in leachates of old rocks), equation (1) reduces to *f_L* = 1 - *R_w/R_p*.

Table 1 Results of ^{36}Cl measurements in samples from the Jordan River/Dead Sea System

Map location no.	Sample origin and collection date	$^{36}\text{Cl}/\text{Cl}^*$ (10^{-15})	$^{36}\text{Cl}/\text{Cl}^\dagger$ (10^{-15})	Cl (mg l^{-1})	^{36}Cl (10^6 atoms l^{-1})	$F_L^\ddagger$	$\text{EF}^\ddagger$	$\text{EF}^\S$ (hydro.)
1	Mount Hermon Snow (January 1983)	1,470 $\pm$ 250 1,425 $\pm$ 300 1,630 $\pm$ 250 1,680 $\pm$ 350 1,400 $\pm$ 300 2,075 $\pm$ 350	1,580 $\pm$ 120	1.50	40 $\pm$ 5			
2	Banias River (July 1983)	440 $\pm$ 140 430 $\pm$ 80 310 $\pm$ 140 340 $\pm$ 150	400 $\pm$ 60	11.9	80 $\pm$ 15	0.74	0.50	0.40
3	Snir River (July 1983)	400 $\pm$ 175 460 $\pm$ 180	430 $\pm$ 125	11.0	80 $\pm$ 20	0.72	0.50	
4	Dan River (July 1983)	725 $\pm$ 140	725 $\pm$ 140	10.5	129 $\pm$ 25	0.54	0.31	
5	Lake Kinneret (April 1983)	49 $\pm$ 15	49 $\pm$ 15	252	210 $\pm$ 65	0.97	0.20	0.25
6	Yarmuq River (July 1983)	186 $\pm$ 48	186 $\pm$ 48	137	431 $\pm$ 110	0.88	0.09	0.16
7	Jordan River (February 1982)	137 $\pm$ 35 114 $\pm$ 22	121 $\pm$ 19	646	1,320 $\pm$ 210	0.92	0.03	0.15
8	Dead Sea 10 m depth (March 1977) 80 m depth (March 1977)	16 $\pm$ 7 27 $\pm$ 9 13 $\pm$ 7 26 $\pm$ 7 21 $\pm$ 6 13 $\pm$ 4 14 $\pm$ 3 48 $\pm$ 12 12 $\pm$ 12 13 $\pm$ 5 29 $\pm$ 10 38 $\pm$ 10	17 $\pm$ 4 17 $\pm$ 2 20 $\pm$ 4	 2.30 $\times 10^5$	 (6.6 $\pm$ 0.8) $\times 10^4$	 0.99		
9	Ashlag Spring (August 1982)	4 $\pm$ 2	4 $\pm$ 2	2.6 $\times 10^5$				
10	Mount Sedom¶ 2,000 m depth	3 $\pm$ 3 6 $\pm$ 3 7 $\pm$ 5	5 $\pm$ 2					

* Results of independent measurements for samples of same origin are given when available. Quoted errors include statistical counting errors or random instrumental errors. The error in the absolute value is $\pm 15\%$.

† Weighted mean of the independent measurements (column 3). Quoted error is the variance of the mean.

‡ See text for definition.

§ Ref. 7; see text.

|| Hypersaline spring near Dead Sea shore.

¶ Rock salt core.

The physical meaning of the above equations is illustrated in Fig. 1. In the case of a closed system (no evaporation), all analytical results plotted in the $^{36}\text{Cl}/\text{Cl}$ versus $(1/\text{Cl})$ plane fall on a straight line PL. The value of the rock leachate fraction f_L determines the location of a given point on the line. Evaporation will shift a point horizontally to the left of the line PL by an amount related to the evaporation factor EF. Any additional source of ^{36}Cl (such as ^{36}Cl from the nuclear test pulse) will shift results of measurements in the vertical direction above the line PL.

The estimation of the salt accumulation age in a water body from ^{36}Cl measurements was first attempted by Bonner *et al.*². This age t (yr) satisfies the equation:

$$N_{36} = \frac{I_{36}}{\lambda} (1 - e^{-\lambda t}) \quad (3)$$

where λ (yr^{-1}) is the decay constant, N_{36} the total number of ^{36}Cl atoms present in the water body and I_{36} the annual input of this isotope. We assume that the amount of salt precipitated from the water is negligible. If the water body, as is often the case with saline lakes, receives in addition to the ordinary

observable chloride input a significant sub-lacustrine input (from springs emerging below the lake surface and hence difficult to observe) of chloride originating from the leaching of old rocks, then the salt accumulation time of the water body may be determined by combining its ^{36}Cl balance with its stable chloride balance, even if the only information known about the sub-lacustrine source is its $^{36}\text{Cl}/\text{Cl}$ ratio. In this case, the annual ^{36}Cl input to the water can be shown to be the sum of two components: the annual ^{36}Cl input of meteoric origin and that of rock leachate origin. The accumulation age then satisfies the following equation:

$$N_{36} = C_w V_w R_w = \frac{Q_I C_I (R_I - R_L) + C_w V_w R_L / t}{\lambda} (1 - e^{-\lambda t}) \quad (4)$$

where Q is the annual water discharge, V the water body volume, the subscript 'I' designates the input to the water body and the other symbols are as defined above.

To illustrate these applications of ^{36}Cl as a hydrological tracer, we consider the waters of the Jordan River/Dead Sea systems. This system (Fig. 2) is located in the northern part of the Syro-African rift and is composed primarily of the upper Jordan river,

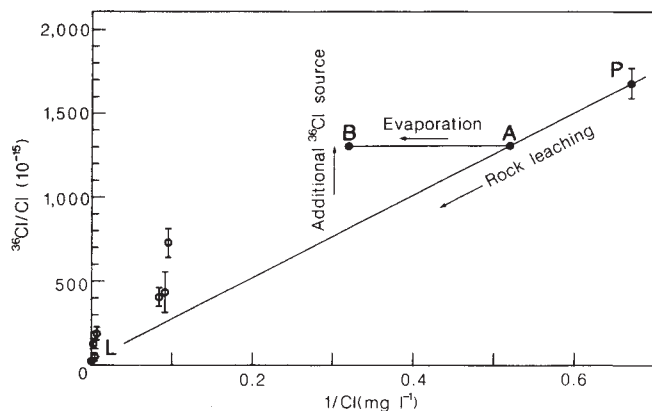


Fig. 1 Variation of the $^{36}\text{Cl}/\text{Cl}$ isotopic ratio versus the reciprocal ($1/\text{Cl}$) of the stable chloride concentration and schematic representation of different effects on the isotopic ratio. The straight line PL represents a hypothetical closed system (no evaporation) in which all samples are admixtures of precipitation (P) and rock leachate (L); as the latter contribution increases, the result moves down along line PL (A). Line AB reflects the effect of evaporation, which does not change the $^{36}\text{Cl}/\text{Cl}$ ratio. Results may lie above the line also due to an additional source of ^{36}Cl (for example, the ^{36}Cl nuclear test pulse). Also shown are results of measurements (open circles and vertical bars) for the Jordan River/Dead Sea system. The fact that all data points lie above the mixing line is mostly explained by evaporation processes (see text).

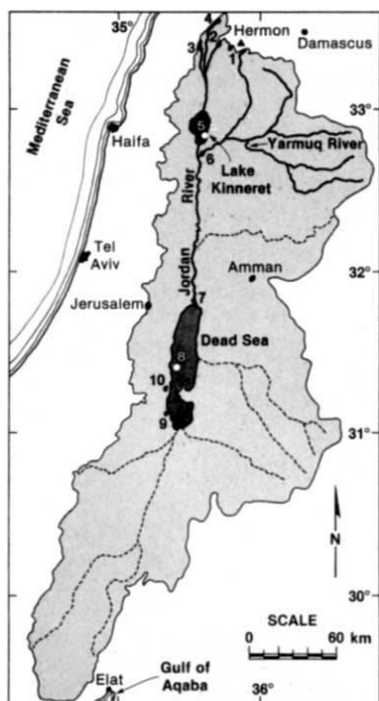


Fig. 2 Map showing the Jordan River/Dead Sea catchment basin (light shading) and the localities (numbered 1–10) sampled for ^{36}Cl measurements. The sample locations and their numbers are listed in Table 1.

Lake Kinneret, the Yarmouk river, the lower Jordan river and the Dead Sea terminal lake. The three important tributaries of the upper Jordan river—the Dan, Snir and Banias rivers—arise as springs which derive their waters from precipitation on Mount Hermon, with an underground residence time of a few years³. These waters collect large quantities of salt from the many saline springs that enter the system during their flow to the Dead Sea. This extremely saline lake acquired its present shape about 15,000 yr ago⁴ as a result of the shrinking of its less saline predecessor, Lake Lisan. This 15,000 yr age is distinct from the

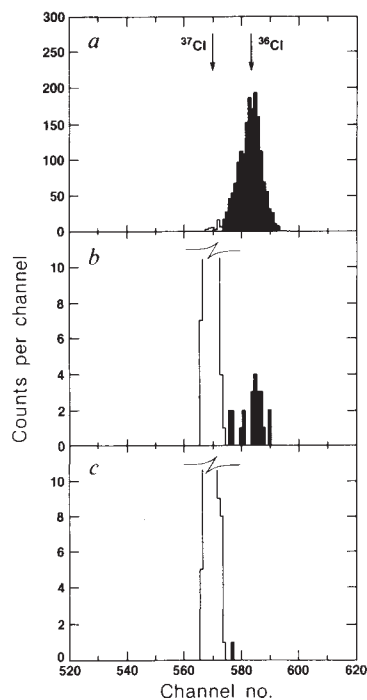


Fig. 3 Identification spectra of chlorine ions for: *a*, reference sample with $^{36}\text{Cl}/\text{Cl} = 3.5 \times 10^{-12}$; *b*, Dead Sea water; *c*, rock salt from Mount Sedom. The spectra were obtained after a software filtering of events to reject background (^{36}S isobaric and contaminant) ions. ^{37}Cl ions shown result from a tail of $^{37}\text{Cl}^-$ injected into the accelerator. They are further discriminated from ^{36}Cl ions by time-of-flight measurements. See ref. 5 for details of the identification scheme.

age of the salt, discussed below. The high salinity of the Dead Sea results partly from intense evaporation and accumulation of the salt discharged by the Jordan River and smaller rivers, and partly from extremely saline springs which emerge at the lake shore and at its bottom.

The water samples on which the present study is based were collected from the localities indicated in Fig. 2. After removing SO_4^{2-} ions with Ba^{2+} , Cl^- was precipitated with Ag^+ and purified by dissolution in NH_3 before the final reprecipitation as AgCl . The $^{36}\text{Cl}/\text{Cl}$ ratio was measured using the accelerator mass spectrometry system set up at the Rehovot 14UD Pelletron tandem laboratory⁵. Figure 3 shows examples of ^{36}Cl spectra. The stable chloride concentrations in the same water samples were determined by titration with AgNO_3 .

The results of our measurements are shown in Table 1 and Fig. 4. The consistent downstream decrease in $^{36}\text{Cl}/\text{Cl}$ in Fig. 4a reflects the progressive dilution of the meteoric ^{36}Cl input, represented by the Mount Hermon snow sample, with the chloride leached from rocks. Samples representing essentially pure rock chloride, whose meteoric ^{36}Cl component is expected to be extinct, were obtained from materials near the Dead Sea: rock salt from an old formation (Mount Sedom) and brine from a hypersaline spring. These samples yielded the two lowest $^{36}\text{Cl}/\text{Cl}$ values in our measurements. These values are, however, higher than ratios determined in some old salt deposits⁶; they may result from natural radioactivity⁶, but at such low levels, experimental effects such as cross-contamination during sample preparation or ion source operation cannot be excluded. The downstream increase in the ^{36}Cl concentration (Fig. 4b), is attributed to evaporation processes. This is also illustrated in Fig. 1 where, in agreement with important evaporation effects, all data points lie above the rock leachate–precipitation mixing line. Extensive evaporation and accumulation are believed to

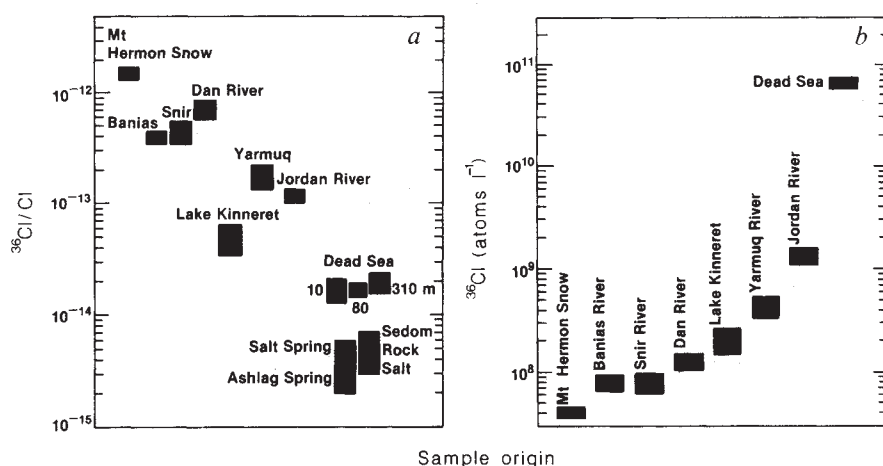


Fig. 4 Systematic variation of ^{36}Cl levels in waters from the Jordan River/Dead Sea system, arranged in downstream order. The distances along the horizontal axis are arbitrary. Also shown are a rock salt sample from a 2,000-m-deep core in the Mount Sedom area and a brine sample from the hypersaline Ashlag spring which is believed to be representative of the sub-lacustrine saline springs: a, $^{36}\text{Cl}/\text{Cl}$ isotopic ratios measured by accelerator mass spectrometry; b, ^{36}Cl concentrations in waters obtained from the $^{36}\text{Cl}/\text{Cl}$ ratios and measurements of the stable chloride concentrations. The monotonous downstream enrichment is attributed mainly to evaporation.

be responsible for the particularly high ^{36}Cl concentration in the terminal lake.

The rock leachate fraction f_L and the evaporation factor EF relative to Mount Hermon snow, calculated using equations (1) and (2), are shown for the various waters of the Jordan River/Dead Sea system in Table 1. The last column in Table 1 shows, for comparison, the available EF values obtained by hydrological methods⁷. Except for the Jordan River, the agreement between the two sets of estimates is surprisingly good. The agreement with independent measurements of the evaporation factors indicates that the additional ^{36}Cl source from the nuclear test pulse, known to have been important around 1960 (ref. 8), does not significantly affect our results. Substituting for example the hydrological estimates (EF(hydro.), Table 1) for Lake Kinneret and the Yarmuq River in equation (2), we derive expected values for the $^{36}\text{Cl}/\text{Cl}$ ratio of 38×10^{-15} and 108×10^{-15} respectively, only marginally different from the measured values, considering the uncertainties in the $^{36}\text{Cl}/\text{Cl}$ and EF(hydro.) values. The discrepancy for the case of the Jordan River may be due in part to such a contribution but is most probably caused also by an increase of evaporation following the great reduction in the flow of the Jordan river (presently <10% of the 1964 flow) which has resulted from the diversion of both Lake Kinneret and Yarmuq River waters for irrigation purposes.

The considerable amount of chloride contributed by rocks, even in the very dilute Jordan river tributaries, could not have been determined previously because it was not possible to distinguish between the effects of evaporation and of rock chloride addition. The relatively high evaporation factors for these tributaries are almost entirely caused by evapotranspiration, whereas the much lower values for the Yarmuq River must result from the intense evaporation which occurs along the long desert route between its source and the sampling point. Finally, the high rock leachate fraction for both Lake Kinneret and the Dead Sea undoubtedly reflect the well-known salt contributions by sub-lacustrine springs⁹. Note that an added advantage of the present method of determining the rock leachate fraction and the evaporation factor over the standard hydrological methods is that the former can be applied to any element of a river system and related directly to precipitation without making any measurements on other water bodies in the system.

Turning now to the determination of the salt accumulation age of the Dead Sea, we first use equation (3). But, as explained above, because of man-made changes in the hydrological system, the ^{36}Cl input of the natural system before 1964 cannot be directly measured. The annual ^{36}Cl input ($I_{36} = 3.9 \times 10^{20}$ ^{36}Cl atoms yr^{-1}) may be estimated using the annual rainfall over the entire Dead Sea basin (9.8×10^{12} l yr^{-1} , ref. 7) and the ^{36}Cl concentration measured in the Mount Hermon snow (Table 1), assuming that it is representative of the whole basin. The number of ^{36}Cl atoms in the Dead Sea, as calculated by the expression

$N_{36} = V_w C_w R_w$, is 9.4×10^{24} ^{36}Cl atoms, using both the Dead Sea volume ($V_w = 1.43 \times 10^{14}$ l, ref. 10) and our measured Cl concentration and $^{36}\text{Cl}/\text{Cl}$ ratio (Table 1). The accumulation time of ^{36}Cl in the Dead Sea thus obtained is 25,000 yr. A value of 20,000 yr is obtained if one uses actual measured contributions of the two main inputs, Lake Kinneret and the Yarmuq River (0.5×10^{12} and 0.44×10^{12} l yr^{-1} , respectively, ref. 7) to estimate the annual ^{36}Cl input; to these contributions we added the annual ^{36}Cl input from precipitations over the rest of the basin (5.1×10^{12} l yr^{-1} , ref. 7), estimated as was done above for the entire basin.

The above calculations assume that the sub-Dead Sea saline springs derive their salt from very old deposits ($R_L = 0$). If we assume that the measured $^{36}\text{Cl}/\text{Cl}$ value of 4.5×10^{-15} in Ashlag Spring and Mount Sedom (Table 1) is a representative value of the rock leachate fraction, we obtain, solving equation (4) by iteration, a range of slightly lower accumulation ages, 19,000–21,000 yr. For this calculation, an effective value of the $^{36}\text{Cl}/\text{Cl}$ ratio of the input waters from the various sources ($R_L = 35\text{--}44 \times 10^{-15}$) was derived by dividing the annual ^{36}Cl input, estimated above, by the Cl discharge (using $Q_L = 1.4 \times 10^{12}$ l yr^{-1} , ref. 7 and $C_L = 474$ mg l^{-1} , ref. 10). This range of R_L values, lower than the $^{36}\text{Cl}/\text{Cl}$ values measured in Lake Kinneret and the Yarmuq River, shows the effect of the known important salt addition in the lower Jordan River basin. The ratio of the ^{36}Cl contributions from meteoric input to that of rock leachate in the total ^{36}Cl content of the Dead Sea water can be estimated to be ~4:1 by using equation (4) and the calculated accumulation age. The contribution of the increase in the ^{36}Cl content of the atmosphere during the nuclear test period⁸ is quite small compared with the amount of ^{36}Cl accumulated in the Dead Sea. The correction to the above accumulation times can be estimated by using the measured integral ^{36}Cl bomb pulse⁸ to be ~500 yr. Furthermore, the contribution of ^{36}Cl produced by the capture of cosmic-ray neutrons by ^{35}Cl in the Dead Sea can be shown to be negligible.

Note that the present ages, although not very much higher than the 12,100 yr geochemical age derived by Bentor¹⁰, were obtained without making any assumptions as to the detailed chemical nature of the saline springs. A comparison of these age estimates with the ~15,000 yr age of the present Dead Sea basin⁴ may indicate that some of the salt now in the Dead Sea was inherited from its predecessor Lake Lisan.

This work was supported in part by the US-Israel Binational Science Foundation and by the Minerva Foundation.

Received 23 December 1985; accepted 21 March 1986.

1. Elmore, D. *et al.* *Nature* **277**, 22–25 (1979).
2. Bonner, F. T. *et al.* *Geochim. cosmochim. Acta* **25**, 261–266 (1961).
3. Simpson, B. & Carmi, I. *J. Hydrol.* **62**, 225–242 (1983).
4. Vogel, J. C. & Waterbolk, H. T. *Radiocarbon* **14**, 6–110 (1972).
5. Fink, D. *et al.* *Nucl. Instrum. Meth.* **B5**, 123–128 (1984).

6. Bentley, H. W., Phillips, F. M. & Davis, S. N. in *Handbook of Environmental Isotope Geochemistry*, Vol. 2 (eds Fritz, P. & Fontes, J. C.) (Elsevier, Amsterdam, in the press).
7. Dalin, Y. *Proc. Symp. Hydrological Activities Dictated by the Energy Crisis*, 5 (Israel National Council for Research and Development, Rehovot, Israel, 1981) (in Hebrew).
8. Elmore, D. *et al. Nature* **300**, 735–737 (1982).
9. Mazor, E., Mero, F. J. *Hydrol.* **7**, 318–333 (1969).
10. Bendor, Y. K. *Geochim. cosmochim. Acta* **25**, 239–260 (1961).

Phosphate pumps and shuttles in the Black Sea

Gary Shaffer

Oceanographic Institute, University of Gothenburg, Box 4038, 400 40 Gothenburg, Sweden

Phosphate distributions in oxic–anoxic ocean basins are related to distributions of total oxygen consumed (mainly from free oxygen, nitrate and sulphate) in the decomposition of organic matter^{1,2}. Data from the Black Sea show, however, that phosphate concentrations immediately above and below the redox front are considerably lower and higher, respectively, than would be predicted from such stoichiometric models. This implies extra sinks and/or sources of phosphate and/or total oxygen not associated with organic decomposition. Here, I present two physical–biogeochemical interactions that result in phosphate sinks and sources with the structure needed to explain the observed phosphate anomalies. A phosphate pump based on iron can account for up to 40% of the required sink–source strength. The rest may be caused by a phosphate shuttle based on manganese.

The salinity, temperature, PO_4^{3-} , NO_3^- , O_2 , H_2S , Fe^{2+} and Mn^{2+} data analysed here originated from cruise 49 of RV *Atlantis II* in March and April 1969 (refs 3, 4). I chose 18 stations which had a good coverage of the Black Sea and good sampling depth and salinity data. All PO_4^{3-} and sample depth data from these stations have been plotted as functions of salinity (Fig. 1) and fitted with least-mean-square B-splines⁵. The splines, plotted against each other, yield a pseudo-mean distribution with depth (PO_4 , Fig. 2). This procedure is superior to conventional depth averaging⁶ or depth averaging relative to an oxygen zero boundary^{4,7}. It averages over isopycnal surfaces, where the salinity determines the density below the Black Sea surface layer, and thus should retain maximum information on mean 'vertical' structure⁸. Indeed, the PO_4 minimum/maximum pair (Fig. 2) reflects a similar structure from detailed, individual PO_4^{3-} profiles from this data set^{6,9}.

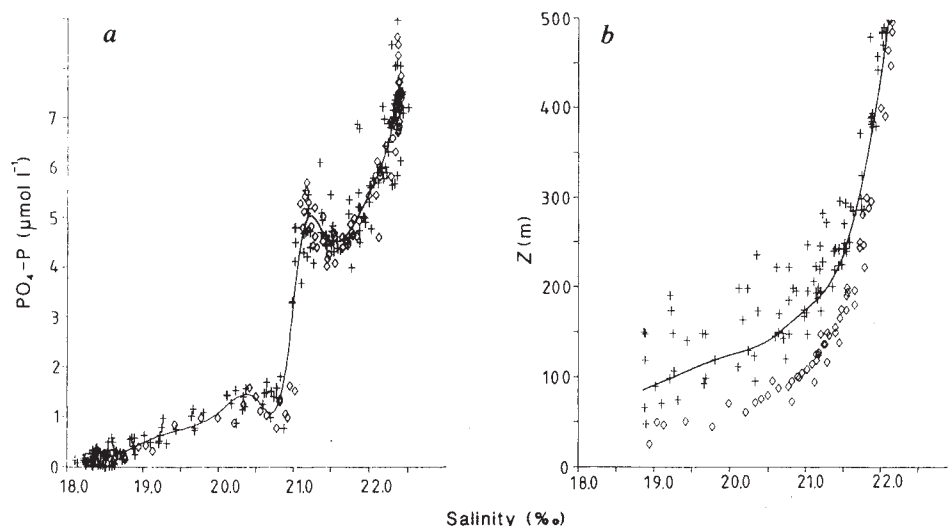
Phosphate distribution can be calculated from organic decomposition, $(\text{PO}_4)_{\text{exp}}$ (Fig. 2) and be expressed as

$$(\text{PO}_4)_{\text{exp}} = a\Delta\text{O}_2 + b\Delta\text{NO}_3 + c\Delta\text{SO}_4 + (\text{PO}_4)_p \quad (1)$$

where $\Delta\text{O}_2 = \text{O}_2(\text{saturation}) - \overline{\text{O}_2}$, $\Delta\text{NO}_3 = d\Delta\text{O}_2 + (\text{NO}_3)_p - \overline{\text{NO}_3}$, and $\Delta\text{SO}_4 = \text{H}_2\text{S}$. The subscript 'p' refers to preformed substances^{1,2}. Here the concentrations of $(\text{NO}_3)_p$ and $(\text{PO}_4)_p$ are 0 and $0.15 \mu\text{mol l}^{-1}$ respectively. Pseudo-mean distributions are determined as above and a – d are $(138)^{-1}$, $(85)^{-1}$, $(53)^{-1}$ and $(8.6)^{-1}$, respectively². $(\text{PO}_4)_{\text{exp}}$ over-estimates $(\text{PO}_4)_{\text{ox}}$ (a 'true' PO_4^{3-} distribution from organic decomposition) above L, the O_2 – H_2S boundary (Fig. 2) and under-estimates it below this boundary. In the present-day Black Sea nearly all H_2S –S produced during sulphate reduction below L should reappear, after advection, diffusion and a series of intermediate oxidation steps, as SO_4^{2-} –S above L. This implies extra O_2 and H_2S consumption, above and below L, respectively, without concurrent PO_4^{3-} release. A $(\text{PO}_4)_{\text{ox}}$ distribution (Fig. 2) based on estimates of this extra O_2 consumption and $(\text{PO}_4)_{\text{ox}} \rightarrow \text{PO}_4$ away from L, underlines some inherent weaknesses in using only chemical data. With a knowledge of diapycnal advection and diffusion, rates could be calculated and a stoichiometric model applied directly to them⁸. The large phosphate anomaly dipole pivoted near the O_2 – H_2S boundary in the Black Sea, however, needs extra sinks and sources of PO_4^{3-} to be explained.

Iron-oxhydroxo-phosphates (FeOP) form in oxidizing conditions ($E_h > \sim 200 \text{ mV}$) and dissolve in reducing conditions ($E_h < \sim 200 \text{ mV}$)^{10,11}. Any combination of physical processes which lead to a net transport of such particles down through the redox front and Fe^{2+} and PO_4^{3-} ions back up through it, would produce an extra sink–source pair for PO_4^{3-} of the type needed to explain the observed anomaly dipole. Two possible cycles containing such combinations are the phosphate pump and the phosphate shuttle (Fig. 3). In the pump, vertical isopycnal displacements sweep the redox front back and forth across a sea-bottom segment B. During the release phase (shallow redoxocline) PO_4^{3-} and Fe^{2+} leave B as FeOP dissolves. Some Fe^{2+} reprecipitates as iron sulphide (FeS). During the retention phase (deep redoxocline) FeS dissolves and FeOP forms on B. PO_4^{3-} is adsorbed from the water column and the pore water. Increased upward diffusion of PO_4^{3-} (caused by its enhanced vertical gradients) closes the cycle. Thus, baroclinic motions near the coast pump PO_4^{3-} down through the O_2 – H_2S boundary in oxic–anoxic basins. In the shuttle, Fe^{2+} is advected and diffused up through the redox front to form FeOP, thereby adsorbing PO_4^{3-} . FeOP particles sink through the front, dissolve, and release PO_4^{3-} . Again increased diffusion closes the cycle.

Fig. 1 Phosphate-phosphorus (a) and water sample depth (b) plotted against salinity for 18 stations of the *Atlantis II* cruise 49 to the Black Sea, March–April 1969 (ref. 3). The open sea stations ($\diamond$) are numbers 1445, 1446, 1447, 1466, and 1468. The shelf stations ($+$, water depth $< 1,800 \text{ m}$) are numbers 1435, 1437, 1439, 1441, 1443, 1447, 1450, 1452, 1454, 1461, 1470, 1473 and 1476. Least mean square B-spline fits⁵ are shown for data from all 18 stations. For the water sample depths linear interpolation in salinity space⁸ was applied to individual stations before B-spline fitting.



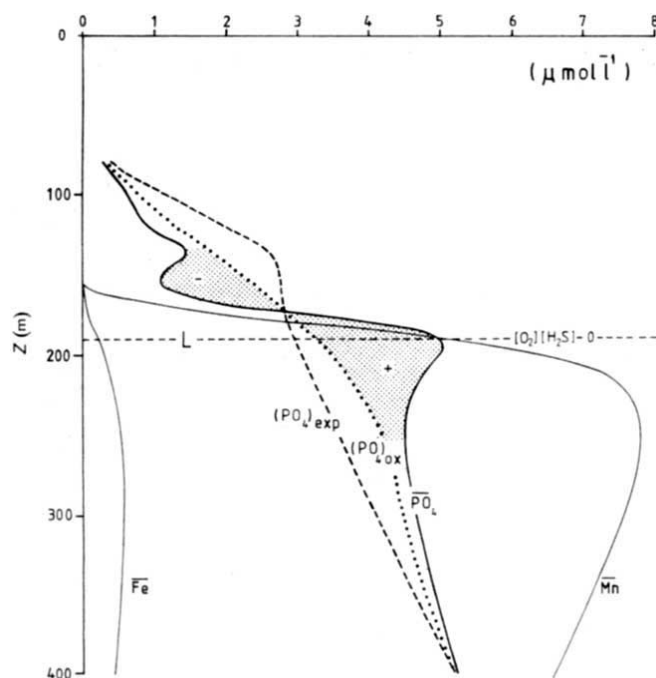


Fig. 2 Pseudo-mean distribution of phosphate-phosphorus with depth, $\overline{PO_4}$, formed by plotting the B-splines in Fig. 1 against each other. This procedure should retain a maximum of 'real' mean vertical structure and gradients. Analogous distributions are shown for dissolved manganese, Mn, and iron, Fe, from data of stations 1435, 1439, 1445, 1446, 1452, 1461, 1464 and 1466 (ref. 4). The zero oxygen and hydrogen sulphide concentration level, L , is indicated (taken from O_2 and H_2S distributions calculated as $\overline{PO_4}$ above). Also presented is the phosphate-phosphorus distribution expected from organic decomposition alone, $(PO_4)_{exp}$, as calculated from equation (2). $(PO_4)_{ox}$ is $(PO_4)_{exp}$ 'corrected' for extra O_2 consumption as described in the text. The phosphate anomaly dipole, shaded, is defined by $(PO_4)_{ox} - \overline{PO_4}$.

The strength of the extra PO_4^{3-} sinks and sources, $|Q_{PO_4}|$, can be estimated from

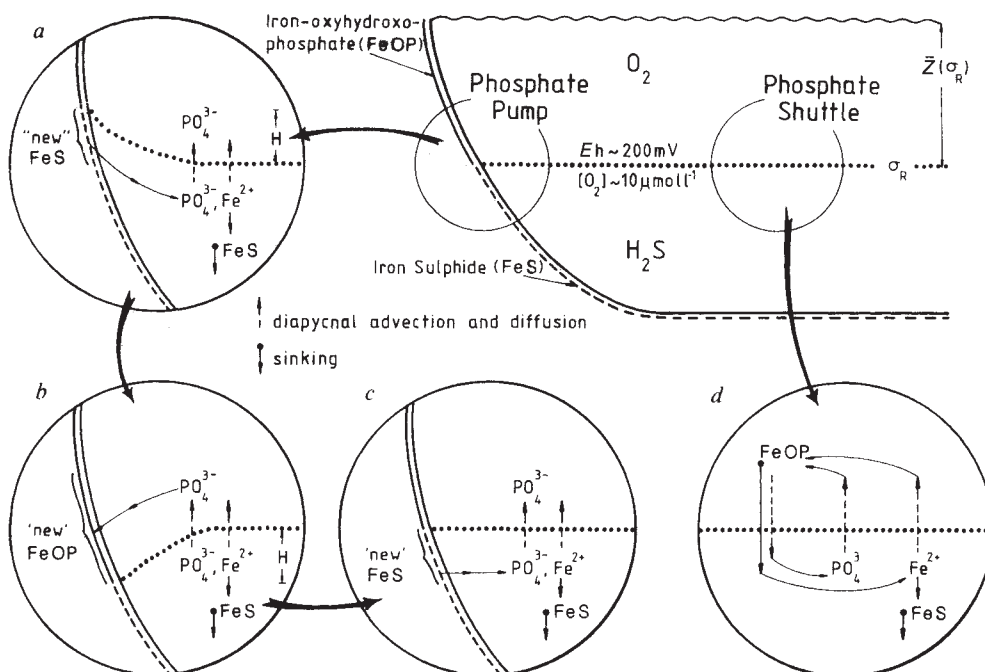
$$Q_{PO_4} \approx Akd(PO_4)_{anom}/dz \quad (2)$$

where A , the cross-sectional area, k , the vertical turbulent diffusion coefficient and the vertical gradient of $(PO_4)_{anom} [(PO_4)_{ox} - \overline{PO_4}]$; all are evaluated at the $(PO_4)_{ox}, \overline{PO_4}$ intersection near level L (Fig. 2). There, $A \approx 3.0 \times 10^{11} \text{ m}^2$ (ref. 12) and $d(PO_4)_{anom}/dz \approx 9 \times 10^{-2} \text{ mmol m}^{-4}$ (Fig. 2). Previous estimates^{4,7} of k of $1.4 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ in the Black Sea halocline are probably in error. Salt conservation requires that the vertical velocity, w , be much greater than the w used to obtain this estimate: A salty (35‰) inflow of $190 \text{ km}^3 \text{ yr}^{-1}$ (refs 4, 13) must entrain much 18.5–22.0% ambient water before becoming 22.0–22.4% deep water; a six to ninefold increase in volume transport (and in the w and k estimates) is implied. Although a constant w and k , one-dimensional model^{4,7} is probably not a good one for a system like the Black Sea, for example, entrainment and decreasing cross-section with depth imply a large increase in w with depth, a k from a salt space method can be estimated^{14,15} which agrees with the new k above and thus the value $k = 1 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ is adopted here; then $|Q_{PO_4}| \approx 2.3 \times 10^{10} \text{ mmol } PO_4^{3-} \text{ day}^{-1}$. A mean cycling time, τ , for PO_4^{3-} ions around the anomaly dipole may be estimated by $|RQ_{PO_4}^{-1}|$ where R is the mean total PO_4^{3-} anomaly. From Fig. 2, $R \approx 1.5 \times 10^{13} \text{ mmol}$ and thus, $\tau \approx 2 \text{ yr}$. A τ of $\sim 500 \text{ yr}$ results for the cycle PO_4^{3-} uptake by phytoplankton followed by PO_4^{3-} regeneration in the deep water.

Isopycnal displacements near the Black Sea coast may be caused by upwelling/downwelling¹⁴, coastal-trapped waves¹⁶ and seasonal changes of the mean circulation¹⁷. The r.m.s. displacement near level L from these processes may be $\sim 30 \text{ m}$, which implies a typical bottom area swept by the phosphate pump of $\sim 1.5 \times 10^{10} \text{ m}^2$ (Fig. 3 and ref. 12) and mean retention-release rates for PO_4^{3-} bound with Fe oxyhydroxides of $\sim 1.5 \text{ mmol m}^{-2} \text{ day}^{-1}$ to explain the anomaly dipole by the pump. Retention-release rates of $0.7\text{--}1.5 \text{ mmol m}^{-2} \text{ day}^{-1}$ have been obtained in benthic chamber experiments meant to simulate redox turnovers^{18,19}. Such high retention rates, however,

Fig. 3 The basic situation shown in the upper right hand corner is a basin with oxygen in the surface water and hydrogen sulphide in the deep water. The redox front between these two layers is represented by an isopycnal surface σ_R with mean depth $\bar{Z}$, chosen to coincide with $Eh \sim 200 \text{ mV}$, the approximate potential of the $Fe^{2+}\text{--}Fe^{3+}$ redox pair. Iron-oxyhydroxo-phosphate (FeOP) and iron sulphide (FeS) are found at the sediment surface in contact with the upper and lower layer respectively. In the phosphate pump, isopycnal displacements sweep back and forth across a segment of sea bottom between depths $\bar{Z} \pm H$. The release and retention phases for PO_4^{3-} are illustrated by insets *a*, *c* and *b*, respectively. The + and - anomalies for PO_4^{3-} should have comparable vertical scales, $\sim 2H$. In the text, it is argued that the efficiency of the pump is limited by its retention phase, also, since some Fe^{2+} is continually lost to the deep anoxic bottoms with FeS, new Fe from runoff is needed to keep the pump primed.

In the phosphate shuttle (*d*), the vertical scales of the + and - anomalies depend on the balance between diapycnal advection and mixing and speeds of sedimentation and chemical reactions. Thus, these scales should differ.



were observed at water column $\text{PO}_4^{3-} \sim 10$ times greater than the Black Sea ones and in artificial flow conditions. Some PO_4^{3-} retention in the sediment is caused by adsorption from the water column. A likely maximum rate for this would be $D[\text{PO}_4^{3-}] \delta^{-1}$ where D is a molecular diffusion coefficient, $[\text{PO}_4^{3-}]$ is the water column PO_4^{3-} and δ is the thickness of the diffusive sublayer at the sediment/water interface. With $D \sim 6 \times 10^{10} \text{ m}^2 \text{ s}^{-1}$ (ref. 20, $T \sim 8^\circ \text{C}$), $[\text{PO}_4^{3-}] \sim 2 \mu\text{mol l}^{-1}$ and $\delta \sim 2 \times 10^{-4} \text{ m}$ (ref. 21, large flow velocities), this rate is $\sim 0.5 \text{ mmol m}^{-2} \text{ day}^{-1}$. The rest of PO_4^{3-} retention is caused by direct adsorption of PO_4^{3-} released in the sediment during organic decomposition. An estimate of the sedimentation rate of organic P is $[w(\text{PO}_4)_{\text{ox}} + k d(\text{PO}_4)_{\text{ox}}/dz]_{\text{L}}$ from a physical transport-production balance. With k as before, $[(\text{PO}_4)_{\text{ox}}, d(\text{PO}_4)_{\text{ox}}/dz]_{\text{L}}$ from Fig. 2 and $w = 1.2 \times 10^{-7} \text{ m s}^{-1}$ in accordance with the discussion earlier, this rate is $\sim 0.06 \text{ mmol m}^{-2} \text{ day}^{-1}$. Thus the efficiency of the phosphate pump seems to be limited by its retention phase and the pump can only explain up to 40% of the Black Sea PO_4^{3-} anomaly dipole.

The strength of the Fe^{2+} sink resulting from precipitation above level L may be estimated from

$$Q_{\text{Fe}} \approx A[w\overline{\text{Fe}} + kd(\overline{\text{Fe}})/dz]_{\text{L}} \quad (3)$$

With A , w , k as above and $[\overline{\text{Fe}}]_{\text{L}}$ from Fig. 2, $Q_{\text{Fe}} \approx 2 \times 10^9 \text{ mmol Fe day}^{-1}$. Thus $Q_{\text{Fe}}/Q_{\text{PO}_4^{3-}} < 0.1$ and a phosphate shuttle based on iron probably makes no significant contribution to the observed PO_4^{3-} anomalies. Concentrations of dissolved manganese are ~ 10 times greater than those of iron in the Black Sea (Fig. 2) caused by the greater solubility of Mn salts in reducing conditions⁴. From a calculation like equation (3), $Q_{\text{Mn}} \approx 5.2 \times 10^{10} \text{ mmol Mn day}^{-1}$ and $Q_{\text{Mn}}/Q_{\text{PO}_4^{3-}} \approx 2.3$. Note that, from equations (2) and (3), $\overline{\text{Fe}}$ and $\overline{\text{Mn}}$, the Q ratios above are insensitive to choices of w and k .

I suggest that manganese, like iron, forms oxyhydroxophosphate and that a shuttle based on Mn explains the rest of the PO_4^{3-} anomaly dipole. This would require a P/Mn ratio of at least 0.3 in the Mn precipitate observed above level L in the Black Sea²². The hypothesis is supported by the $\overline{\text{Mn}}$, $\overline{\text{PO}_4}$ and $(\text{PO}_4)_{\text{ox}}$ distributions (Fig. 2) which indicate good spatial correlations between Mn^{2+} and extra PO_4^{3-} sinks and sources. For both ions, the scale of the source layer is apparently greater than that of the sink layer. This is consistent with the shuttle scenario (Fig. 3). Also, the PO_4^{3-} anomalies appear more pronounced for open Black Sea stations (Fig. 1), and may indicate an open sea process. The PO_4 distribution (Fig. 2) and our w and k imply an order of magnitude weaker PO_4^{3-} sink directly below the PO_4 maximum. This has been attributed to bacterial chemosynthesis⁷.

Similar large PO_4^{3-} anomalies near the O_2 - H_2S zone have been observed elsewhere, for example, in Lake Nitinat². The processes presented here probably also explain these observations, and detailed sampling should reveal similar PO_4^{3-} anomaly dipoles in most other oxic-anoxic basins. Finally, observational and theoretical searches for other possible important effects should be carried out, for instance, on denitrification and sulphate reduction rates, of redox front bottom sweeping due to coastal dynamics. After all, such physical processes not only expose the sediment surface to cyclic changes of O_2 and H_2S concentrations but also may alter the diffusive sublayer thickness in a systematic way through associated currents.

I thank David Dyrssen for help with the data and literature search and for discussions. This research was supported by grants from the geoscience division of the Swedish Natural Science Research Council.

Received 28 October 1985; accepted 7 March 1986.

1. Richards, F. A. in *Proc. 2nd Int. Wat. Pollut. Res. Conf.* Tokyo 1964, 215-243 (Pergamon, Oxford, 1965).
2. Richards, F. A. in *Chemical Oceanography* Vol. 1, (eds Riley, J. P. & Skirrow, G.) 611-645 (Academic, London, 1965).
3. Brewer, P. G. *Woods Hole Oceanogr. Inst. Tech. Rep. Ref. no. 71-65* (1971).

4. Spencer, D. W. & Brewer, P. G. *J. geophys. Res.* **76**, 5877-5892 (1971).
5. Cox, M. G. *J. Inst. Math. Appl.* **10**, 134-149 (1972).
6. Fonselius, S. H. in *The Black Sea—Geology, Chemistry and Biology* (eds Degens, E. T. & Ross, D. A.) 144-150 (American Association of Petroleum Geologists, Tulsa, 1984).
7. Brewer, P. G. & Murray, J. W. *Deep-Sea Res.* **29**, 803-818 (1973).
8. Shaffer, G. & Rönner, U. *Deep-Sea Res.* **31**, 197-220 (1984).
9. Grasshof, K. in *Chemical Oceanography* Vol. 2 (eds Riley, J. P. & Skirrow, G.) 455-597 (Academic, London, 1975).
10. Einsele, W. *Archiv. Hydrobiol. Plankton* **33**, 361-387 (1938).
11. Krom, M. D. & Berner, R. A. *Geochim. cosmochim. Acta* **45**, 207-216 (1981).
12. Deuser, W. G. in *The Black Sea—Geology, Chemistry, and Biology* (eds Degens, E. T. & Ross, D. A.) 133-136 (American Association of Petroleum Geologists, Tulsa, 1974).
13. Merz, A. & Möller, L. *Veröff. Inst. Meeresforsch. Berlin. Neue Folge* A18 (1928).
14. Shaffer, G. *J. Phys. Oceanogr.* **9**, 847-855 (1979).
15. Walin, G. *Tellus* **29**, 128-136 (1977).
16. Csanady, G. T. *Circulation in the Coastal Ocean* (Reidel, Dordrecht, 1982).
17. Neumann, G. *Z. Ges. Erdk. Berl.* **3**, 92-114 (1944).
18. Balzer, W., Grasshoff, K., Dieckmann, P., Haardt, H. & Petersohn, U. *Oceanol. Acta* **6**, 337-344 (1983).
19. Holm, N. *Ophelia* Suppl. 1, 287-304 (1978).
20. Li, Y.-H. & Gregory, S. *Geochim. cosmochim. Acta* **38**, 703-714 (1974).
21. Santschi, P. H., Bower, P., Nyffeler, U. P., Azevedo, A. & Broecker, W. S. *Limnol. Oceanogr.* **28**, 899-912 (1983).
22. Spencer, D. W., Brewer, P. G. & Sachs, P. L. *Geochim. cosmochim. Acta* **36**, 71-86 (1972).

Mechanosensitivity of mammalian auditory hair cells *in vitro*

I. J. Russell, G. P. Richardson & A. R. Cody

MRC Neurophysiology Group, School of Biology, University of Sussex, Falmer, Brighton BN1 9QG, UK

Intracellular responses recorded *in vitro* from the cochleas of anaesthetized mammals have shown that the mechanoreceptive inner and outer hair cells are sharply tuned, accounting for many of the properties of the afferent fibres in the auditory nerve¹⁻⁴. However, *in vivo* it has not been possible to measure directly the excitatory mechanical input to these cells (the displacement of their mechanosensitive stereocilia)^{5,6} and thus to determine the relationship between the receptor potentials and displacement of their stereocilia. As a means of circumventing this technical difficulty, we have developed an organ culture of the mouse cochlea and here we describe the receptor potentials generated by the hair cells in response to direct displacement of their stereocilia.

Cochleas were dissected from mice at days 1-3 postnatal. The capsules surrounding the cochleas, stria vascularis and Reissner's membranes were removed and the organs of Corti, together with the spiral ganglia, were cultured for 2-7 days at 37°C on collagen-coated coverslips in Maximow slide assemblies using a method similar to that described by Sobkowicz *et al.*⁷. Figure 1a, b shows the morphology of the hair cells in these *in vitro* preparations. Figure 2 shows the arrangement for making intracellular recordings from the hair cells while displacing their stereocilia. The resting membrane potentials of inner hair cells, outer hair cells and supporting cells measured *in vitro* in HEPES-buffered HBSS (Hanks' balanced salt solution) are very similar to those found *in vivo* for the cells of the guinea pig cochlea: -30 to -70 mV for inner hair cells, -40 to -70 mV for outer hair cells and -60 to -75 mV for supporting cells. In contrast to the situation *in vivo*, there is no endocochlear potential in these cultures and the abneural surfaces of the hair cells are exposed to an ionic environment whose dominant cation is sodium rather than potassium.

In the absence of mechanical stimulation, the resting membrane potentials of the hair cells were remarkably constant and did not exhibit the oscillatory behaviour characteristic of hair cells in the turtle cochlea and frog sacculus^{8,9}. The voltage responses of inner and outer hair cells from the same region of the mouse cochlea to sinusoidal displacement of their stereocilia (Fig. 3a, b) were very similar in shape to those recorded from hair cells in isolated preparations from other species (for example, bullfrog sacculus⁵). The transfer functions in Fig. 3c, d show that in response to depolarizing displacements of their stereocilia towards the kinocilium, the responses of the inner

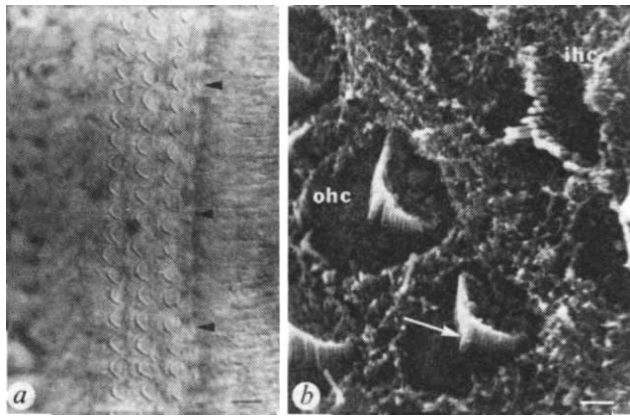


Fig. 1 *a*, Surface view of a 4-day-old culture of a mouse cochlea viewed using Nomarski optics. The stereocilia of the three rows of outer hair cells are clearly visible. The position of the single row of inner hair cells is marked by the arrowheads. The stereocilia of the inner hair cells are positioned slightly higher than those of the outer hair cells and are out of focus in this photomicrograph. The stereocilia of the inner hair cells form palisades while those of the outer hair cells form V-shapes whose internal angles become increasingly acute towards the basal high-frequency turn of the organ of Corti. Scale bar, 10 μm . *b*, Scanning electron micrograph of a 2-day-old organ culture of the mouse organ of Corti, showing the arrangement of the mechanically sensitive stereocilia of the inner (ihc) and outer (ohc) hair cells. The cells still possess a vestigial kinocilium (arrow) which is lost later in development. In scanning electron micrographs the bundles of stereocilia have the appearance of bundles of organ pipes as each row decreases in height with distance from the kinocilium. In these immature hair cells the stereocilia are more numerous than those found in the adult, and the microvilli on the apical surfaces of the intercalated supporting cells are more extensive. Scale bar, 1 μm .

Methods. Cultures were prepared from the cochleas of mice at days 1–3 postnatal. The cartilaginous capsule, stria vascularis, Reissner's membrane and the immature mesenchymal internal spiral lamina were removed by dissection in HEPES-buffered HBSS, leaving the organ of Corti, greater epithelial ridge and attached spiral ganglion as one unit. These tissue pieces were then explanted onto collagen-coated round glass coverslips, fed with $\sim 50 \mu\text{l}$ of complete medium (8 parts Eagle's minimum essential medium with Earle's salts, 1 part heat-inactivated horse serum, 1 part mouse embryo extract buffered with 10 mM HEPES pH 7.2) and maintained in Maximow slide assemblies in the lying drop position at 37 °C for up to 7 days. For scanning electron microscopy the cultures were fixed in 5% glutaraldehyde in 0.1 M sodium phosphate, pH 7.2, for 2 h at room temperature, post-fixed in 1% OsO_4 , dehydrated in acetone, dried to the critical point from liquid CO_2 , sputter-coated with gold and viewed with a Jeol 100C scanning transmission electron microscope.

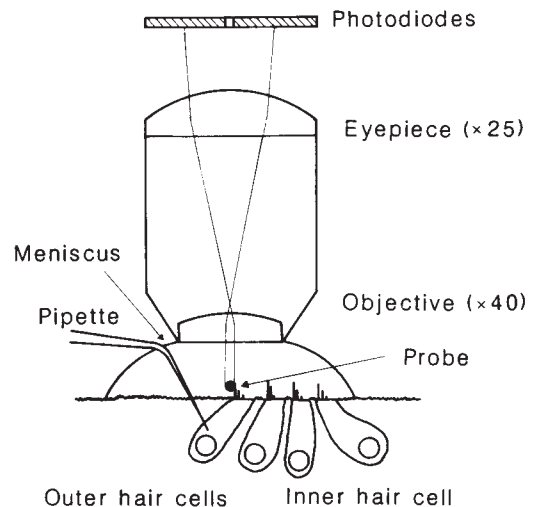


Fig. 2 For the electrophysiological experiments hair cells were maintained at room temperature (20 °C) and observed with the aid of a microscope equipped with a fixed stage, Nomarski optics and a $\times 40$ water immersion lens. The stereocilia of the hair cells were displaced with the tip of a fine glass probe (0.5–1.5 μm diameter) along their axis of greatest sensitivity, that is, towards and away from the kinocilium in a plane parallel to the cuticular plate¹⁷. The probe was attached to a piezoelectric driver constructed from a short length of Vernitron bimorph (type PZT-5B). The displacement of the tip of the probe was monitored according to a method described elsewhere¹⁸. With the preparation illuminated in bright field from a battery-driven light source, the secondary image of the microscope was projected onto a screen by a $\times 25$ eyepiece fitted to a trinocular head stage. The focused image of the tip of the probe was adjusted until it was $\sim 0.5 \text{ mm}$ wide and thus filled about half the width of a double photodiode array (2 $\times$ 0.5 mm; Centronics Ltd 2-5) mounted at the centre of the screen. The differences between the photocurrents of the two photodiodes was proportional to the displacement of the probe tip over the range 1–500 nm. The absolute displacement of the tip was determined as described by Art *et al.*¹⁹. The position of the photodiode array (which was mounted on a micropositioner) was adjusted until the difference in photocurrents was zero; the photodiode array was then displaced by a known amount (1–10 μm) and the photocurrent measured. The same photocurrent would have been measured if the tip had been displaced by this amount divided by the magnification of the optical system ($\sim \times 1,000$). Glass micropipettes (resistance 120–300 M Ω) were bent at right angles 200–250 μm from their tips, filled with 4 M potassium acetate, and inserted into the hair cells close to the nucleus. When stable recording conditions were achieved, the vibrating probe was placed against the stereocilia and the static position of the probe adjusted until a maximum response was obtained for a displacement of $\sim 50 \text{ nm}$.

and outer hair cells are about three times greater than the hyperpolarizing and strongly saturating responses to displacements in the opposite direction. In this respect, the receptor potentials of hair cells in the mouse cochlea *in vitro* are similar to the receptor potentials recorded *in vivo* for hair cells from the apical, low-frequency turns of the guinea pig cochlea in response to tones close to their most sensitive frequencies; they are also similar to receptor potentials recorded for inner hair cells from the basal turn in response to low-frequency tones^{2,10,11}. They differ from the responses of outer hair cells, however, in the basal high-frequency turn of the guinea pig cochlea to very-low-frequency tones, where the receptor potentials are predominantly hyperpolarizing, except at high intensities when they become depolarizing¹². We propose that the different transfer functions of outer hair cells in the basal turn of the guinea pig cochlea may result from their mechanical interaction with the tectorial membrane. In the *in vitro* preparations the tectorial membrane does not cover the hair cells because it has not grown

over them by the stage at which the organs are explanted. Thus, in the *in vitro* preparation, the outer hair cells do not interact with the tectorial membrane.

Figure 3 shows that the receptor potentials of the outer hair cell saturate at a lower voltage than those of the inner hair cell. Results from a further five outer hair cells showed that the receptor potentials saturate in the depolarizing direction at a mean of 6.5 mV (s.d. = 1.4 mV) above resting membrane potential, while the responses of the inner hair cells studied always saturated at higher levels. Further experiments are needed to determine whether the lower saturating voltage levels in outer hair cells reflect smaller receptor currents. The transfer functions of the hair cells shown in Fig. 3 show that the inner hair cells are most sensitive to stereocilia displacements in the depolarizing direction over the 0–60-nm range whereas the outer hair cells are most sensitive over the 0–30-nm range. This measure of sensitivity and dynamic range is largely dependent on the coupling between the stimulus probe and the stereocilia and is

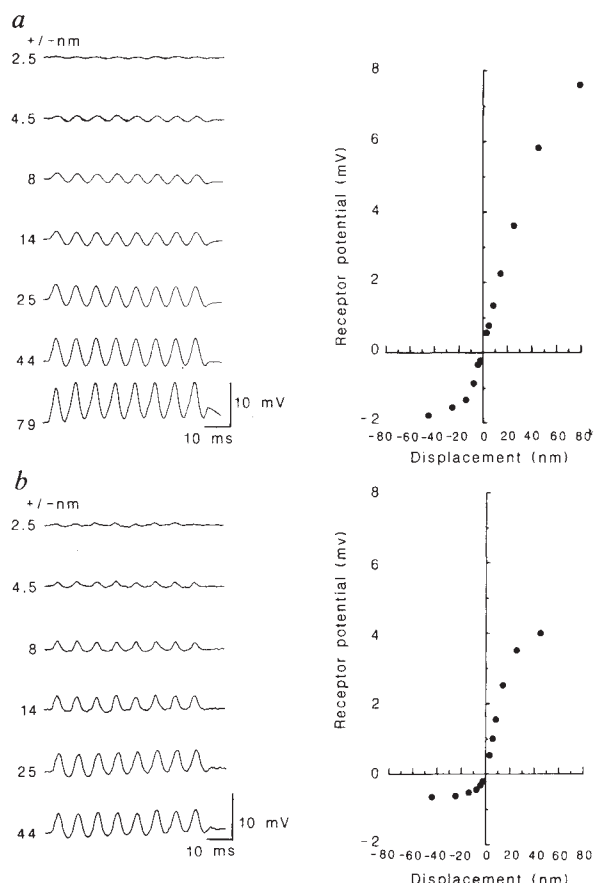


Fig. 3 Intracellular receptor potentials recorded from an inner hair cell (a) and an outer hair cell (b) from the apical low-frequency turn of the mouse cochlea *in vitro*, in response to sinusoidal displacements of their stereocilia. The peak amplitudes of the displacements are indicated alongside each trace. c, d, The relationship between peak displacement of the stereocilia and receptor potential for the inner hair cell and the outer hair cell, respectively.

not necessarily an indication that outer hair cells are more sensitive than inner hair cells. The stereocilia are at most 4 μm long, and it is difficult to determine the exact point of contact of the probe along the length of the stereocilia. Obviously, the angular rotation of the stereocilia will be greatest for a given displacement the closer it is to their points of attachment on the abneural surface of the hair cells.

The most sensitive cells recorded from the mouse cochlea *in vitro* are depolarized by ~ 0.8 mV (mean = 0.53, s.d. = 0.17, $n = 18$) when their stereocilia are displaced by 2 nm (Fig. 3c,d). The tallest hair-cell stereocilia in the *in vitro* preparations are 3–4 μm long. Thus the sensitivity of these hair cells is ~ 30 mV per degree of angular rotation of their stereocilia, or 0.4 mV per nm of displacement. This estimate of hair-cell sensitivity is considerably greater than that of hair cells in the frog sacculus (5–7 mV per μm), based on their responses to displacements of their stereocilia^{5,9}, but similar to estimates of hair-cell sensitivity reported for the turtle cochlea when the influence of the membrane resonance is disregarded (0.2 mV per nm)¹³.

We have compared the mechanosensitivities of hair cells recorded *in vivo* in the basal turn of the guinea pig cochlea with those recorded from the mouse cochlea *in vitro*. Inner hair cells in the basal turn of the guinea pig cochlea are depolarized by 0.8–2 mV in response to tones close to their best frequencies at neural threshold¹. At this sound level the basilar membrane displacement has been estimated to be ~ 0.1 nm¹⁴. In the intact cochlea the tips of the stereocilia are in contact with, or embedded in, the tectorial membrane and they are free to pivot about their bases¹⁵. This mechanical arrangement amplifies the rotational

displacement of the stereocilium caused by the displacement of the basilar membrane by the ratio of the thickness of the organ of Corti to the stereocilium height which, in the basal turn of the guinea pig cochlea, is ~ 10 (ref. 16). Thus, at neural threshold the displacement of the stereocilia is probably ~ 1 nm. In comparing the mechanosensitivities of hair cells in the two preparations, it should be remembered that the driving voltage for the receptor current in hair cells recorded *in vivo* is about 2–3 times that of the hair cells *in vitro*, because the driving voltage is provided by the hair-cell membrane potential (-40 to -80 mV; refs 1, 11) and the endocochlear potential ($+80$ to $+90$ mV), producing a total of 120–170 mV in the guinea pig cochlea *in vivo*. In the mouse cochlea *in vitro*, the driving voltage is produced solely by the hair-cell membrane potential (-30 to -70 mV). Thus, the measurements of the sensitivity of inner hair cells in the mouse cochlea *in vitro* (0.4 mV per nm displacement of their stereocilia) are similar to the estimates of the mechanosensitivity of inner hair cells in the guinea pig cochlea *in vivo*, if the increased driving voltage due to the endocochlear potential is taken into account. In view of this finding, the *in vitro* preparation offers the opportunity for direct measurements of the electrical and mechanical properties of mammalian cochlear hair cells and for elucidation of the mechanisms of mechano-electrical transduction.

We thank Mrs E. M. Cowley, Mr D. Bowell and Dr J. Thorpe for technical assistance, and the MRC for support.

Received 13 February; accepted 10 April 1986.

1. Russell, I. J. & Sellick, P. M. *J. Physiol., Lond.* **284**, 261–290 (1978).
2. Dallos, P., Santos-Sacchi, J. & Flock, A. *Science* **218**, 582–584 (1982).
3. Goodman, D. A., Smith, R. L. & Chamberlain, S. C. *Hearing Res.* **7**, 161–179 (1982).
4. Brown, M. C., Nuttall, A. R. & Masta, R. I. *Science* **222**, 69–72 (1983).
5. Hudspeth, A. J. & Corey, D. P. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2407–2411 (1977).
6. Hudspeth, A. J. & Jacobs, R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1506–1509 (1979).
7. Sobkowicz, H. M., Bereman, B. & Rose, J. E. *J. Neurocytol.* **4**, 543–572 (1975).
8. Crawford, A. C. & Fettiplace, R. *J. Physiol., Lond.* **306**, 79–125 (1980).
9. Ashmore, J. F. *Nature* **304**, 536–538 (1983).
10. Dallos, P. *J. Neurosci.* **5**, 1591–1608 (1985).
11. Russell, I. J. & Sellick, P. M. *J. Physiol., Lond.* **338**, 179–206 (1983).
12. Cody, A. R. & Russell, I. J. *Nature* **315**, 662–665 (1985).
13. Crawford, A. C. & Fettiplace, R. *J. Physiol., Lond.* **364**, 359–380 (1985).
14. Sellick, P. M., Patuzzi, R. & Johnstone, B. M. *J. acoust. Soc. Am.* **72**, 131–141 (1982).
15. Lim, D. J. *J. acoust. Soc. Am.* **67**, 1686–1695 (1980).
16. Johnstone, J. R. & Johnstone, B. M. *J. acoust. Soc. Am.* **40**, 1405–1413 (1966).
17. Shottwell, S. L., Jacobs, R. & Hudspeth, A. J. *Ann. N.Y. Acad. Sci.* **374**, 1–10 (1981).
18. Corey, D. P. & Hudspeth, A. D. *J. Neurosci. Meth.* **3**, 183–202 (1980).
19. Art, J., Crawford, A. C. & Fettiplace, R. *J. Physiol., Lond.* (in the press).

NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones

Amy B. MacDermott*, Mark L. Mayer†, Gary L. Westbrook†, Stephen J. Smith‡ & Jeffery L. Barker†

* Laboratory of Neurophysiology, NINCDS and † Laboratory of Developmental Neurobiology, NICHD, National Institutes of Health, Bethesda, Maryland 20892, USA

‡ Section of Molecular Neurobiology, Yale University School of Medicine, New Haven, Connecticut 06510, USA

Excitatory amino acids act via receptor subtypes in the mammalian central nervous system (CNS)^{1–3}. The receptor selectively activated by *N*-methyl-D-aspartic acid (NMDA) has been best characterized using voltage-clamp and single-channel recording; the results suggest that NMDA receptors gate channels that are permeable to Na^+ , K^+ and other monovalent cations^{4–7}. Various experiments suggest that Ca^{2+} flux is also associated with the activation of excitatory amino-acid receptors on vertebrate neurones^{8–11}. Whether Ca^{2+} enters through voltage-dependent Ca^{2+} channels or

§ Present address: Howard Hughes Medical Institute, Columbia University, CPS, 722 West 168th Street, New York, New York 10032, USA.

through excitatory amino-acid-activated channels of one or more subtype is unclear. Mg^{2+} can be used to distinguish NMDA-receptor-activated channels from voltage-dependent Ca^{2+} channels, because at micromolar concentrations Mg^{2+} has little effect on voltage-dependent Ca^{2+} channels¹² while it enters and blocks NMDA receptor channels^{4,5,7,13,14}. Marked differences in the potency of other divalent cations acting as Ca^{2+} channel blockers compared with their action as NMDA antagonists also distinguish the NMDA channel from voltage-sensitive Ca^{2+} channels^{5,7}. However, we now directly demonstrate that excitatory amino acids acting at NMDA receptors on spinal cord neurones increase the intracellular Ca^{2+} activity, measured using the indicator dye arsenazo III, and that this is the result of Ca^{2+} influx through NMDA receptor channels. Kainic acid (KA), which acts at another subtype of excitatory amino-acid receptor, was much less effective in triggering increases in intracellular free Ca^{2+} .

Experiments were performed using spinal cord neurones in dissociated cultures prepared from 13–14-day-old mouse embryos as described previously^{5–7}. After 1–4 weeks in culture, whole-cell recording was performed using the patch-clamp technique¹⁵ with a high-speed discontinuous voltage-clamp amplifier. The intracellular solution contained 0.8–1.5 mM arsenazo III dissolved in a conventional electrolyte solution for whole-cell recording (see Fig. 1 legend for details). The application of NMDA to spinal cord neurones voltage-clamped at -50 to -60 mV evoked an inward current accompanied by an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) signalled by wavelength-specific changes in the absorbance of arsenazo III (Fig. 1). The inward current activated by NMDA was accompanied by an increase in variance (Fig. 1), and fluctuation analysis (not shown) had the properties characteristic of the 50-pS channel of mean open time 5–6 ms linked to NMDA receptors on mesencephalic, striatal and cerebellar cultured neurones^{4,16}. Under the conditions of our experiments, NMDA-evoked increases in $[Ca^{2+}]_i$ similar to that shown in Fig. 1, were recorded in almost all neurones examined ($n = 29$).

In each neurone a depolarizing voltage jump, usually a step from -60 to 0 mV for 0.5–2 s, was used to induce Ca^{2+} influx through conventional voltage-regulated Ca^{2+} channels to check on the adequacy of the optical recording system before application of excitatory amino acids. Voltage-sensitive Ca^{2+} entry in spinal cord neurones, measured using arsenazo III, could be detected with command voltages as negative as -40 mV, depending on the duration of the step. The increase in $[Ca^{2+}]_i$ peaked near $+10$ mV, and declined in amplitude on further depolarization; at membrane potentials more positive than $+80$ mV Ca^{2+} entry was too small to measure. The similarity of the arsenazo III voltage-absorbance curve to the current-voltage relationship of neuronal Ca^{2+} currents¹⁷ suggests good voltage-clamp control of the membrane area through which Ca^{2+} influx was measured. Such results are similar to those obtained in larger cells^{18,19} and show that arsenazo III can be used to measure Ca^{2+} entry in mammalian spinal cord neurones.

Increases of $[Ca^{2+}]_i$ evoked by excitatory amino acids could be caused by either transmembrane influx of Ca^{2+} or release of Ca^{2+} from intracellular stores. For example, production of inositol trisphosphate ($InsP_3$), one of the second messengers known to stimulate intracellular Ca^{2+} mobilization²⁰, may increase in response to excitatory amino acids²¹. The fact that ion channels activated by excitatory amino acids allow influx of Na^+ provides another indirect mechanism for the increase in $[Ca^{2+}]_i$ induced by NMDA (that is, Na^+ -stimulated Ca^{2+} release from mitochondria²²). We used two procedures (Fig. 2) to demonstrate that transmembrane ion flux is necessary for the appearance of the arsenazo III signal evoked by NMDA, thus eliminating a major contribution by $InsP_3$ production to the elevation in $[Ca^{2+}]_i$ described here. Figure 2a shows the blockade by Mg^{2+} of both the inward current and the increase in $[Ca^{2+}]_i$ evoked by NMDA; as Mg^{2+} ions interfere with ion flux through the NMDA-receptor-activated channel^{4,5,7,13,14}, without

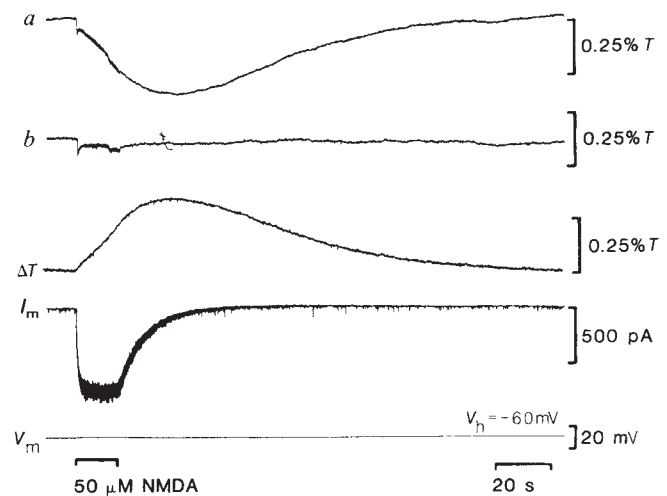


Fig. 1 NMDA increases $[Ca^{2+}]_i$, as measured with the metallo-chrome indicator dye arsenazo III in mouse spinal cord neurones under voltage clamp. Traces *a* and *b* show optical absorbance signals recorded simultaneously at wavelengths of: *a*, 660 nm (the wavelength at which changes in $[Ca^{2+}]_i$ evoke maximal increases in absorbance) and *b*, 570 nm (the isosbestic wavelength of arsenazo III, used to control for movement-related artefacts). Application of 50 μ M NMDA reduced the transmittance at 660 nm but not at 570 nm, indicating an increase in $[Ca^{2+}]_i$. The trace labelled ΔT shows a differential record, obtained by subtracting the transmittance at 660 nm from that at 570 nm, and detects optical absorbance changes due to arsenazo III binding of Ca^{2+} ; an upward deflection reflects an increase in $[Ca^{2+}]_i$. Small deflections in traces *a* and *b* are movement-related artefacts caused by pressure application (90 ms, ≤ 1 p.s.i., 3.6 Hz) of NMDA; these are subtracted in the third trace, the difference record. I_m , Membrane current; application of 50 μ M NMDA evoked an inward current associated with a large increase in variance as described previously⁴. V_m , Membrane potential, voltage-clamped at -60 mV to prevent Ca^{2+} entry through voltage-gated calcium channels. The slow decay of the optical traces probably reflects sequestration and extrusion of calcium ions¹⁹ and is typical of responses recorded using arsenazo III.

Methods. Experiments were performed on the stage of an inverted microscope. Light from a quartz halogen source was focused onto the cell body and an iris diaphragm used to select a field 50 μ m in diameter; spinal cord neurones 15–20 μ m in diameter were centred in this field for whole-cell recording using patch pipettes containing 0.8–1.5 mM arsenazo III. Light from the microscope objective was focused onto a diffraction grating and photodiodes used to measure the intensity at 570, 610, 660 and 700 nm. $[Ca^{2+}]_i$ signals were recorded differentially as 570–660 nm or 700–660 nm. The change in absorbance at 660 nm was greater than that at 610 nm, as expected for changes in $[Ca^{2+}]_i$ rather than intracellular pH. Records are calibrated as changes in transmittance (*T*) from the pre-stimulus baseline. Calibration of optical signals in terms of the underlying free calcium change was not attempted here, because of uncertainties as to dye behaviour in the cytoplasm, and difficulties in estimating the path length and stray light under our experimental conditions. The intracellular solution contained (in mM): 140 K-gluconate, 2 $MgCl_2$ and 10 HEPES titrated to pH 7.2 with KOH; sucrose was added to adjust the osmolarity to 310 mosM. In some experiments CsCl or CsMeSO₃ was substituted for K-gluconate. In addition, the intracellular solution usually contained 1.1 mM EGTA without or with 0.1 mM $CaCl_2$, but similar results were obtained with either no EGTA and no $CaCl_2$ (in this solution arsenazo III chelates residual free Ca^{2+} normally present in patch solutions) or with 5 mM EGTA plus 0.5 mM $CaCl_2$. The extracellular solution contained (in mM): 145 NaCl, 2.5 KCl, 2.5 $CaCl_2$, no added Mg^{2+} , 10 glucose, and 10 HEPES titrated to pH 7.3 with NaOH; sucrose was added to adjust the osmolarity to 325 mosM. Spontaneous activity and synaptic transmission were reduced by adding 1 μ M tetrodotoxin. NMDA, KA and L-glutamic acid were applied to individual spinal cord neurones by minipipette using extracellular patch pipettes.

Experiments were performed at room temperature (25 °C).

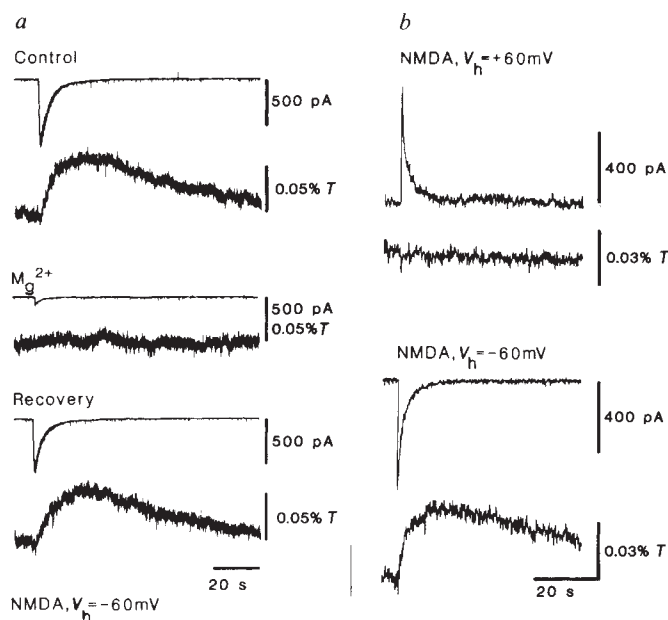


Fig. 2 Increases of $[Ca^{2+}]_i$ result from inward current flow through ion channels activated by NMDA. **a**, Reversible block by pressure application of Mg^{2+} of both the inward current and the increase in $[Ca^{2+}]_i$ evoked by brief application of NMDA (350 ms, 1 mM) to a spinal cord neurone voltage-clamped at -60 mV; an upward deflection represents an increase in $[Ca^{2+}]_i$. The fact that Mg^{2+} ions block the channels activated by NMDA, but do not interfere with agonist binding to the receptor, suggests that ion flux through the NMDA-receptor-activated channel is necessary for the increase in $[Ca^{2+}]_i$. $MgCl_2$ (10 mM) was dissolved in the bathing medium and applied from a patch pipette similar to that used to evoke NMDA responses; the actual concentration of Mg^{2+} at the membrane surface was unknown but dilution by the Mg^{2+} -free bathing medium is expected to have decreased the concentration below that in the pipette. **b**, Responses to pressure application of NMDA (110 ms, 1 mM) recorded at holding potentials of $+60$ mV and -60 mV (the NMDA reversal potential is ~ 0 mV); at $+60$ mV NMDA evokes outward current flow, with no change in $[Ca^{2+}]_i$, whereas at -60 mV NMDA evokes inward current flow associated with an increase in $[Ca^{2+}]_i$. NMDA evokes current flow of similar amplitude at both membrane potentials, suggesting that inward current flow through ion channels activated by NMDA is the source of the increase in $[Ca^{2+}]_i$.

preventing agonist binding²³, this result suggests that $[Ca^{2+}]_i$ is increased by transmembrane ion flux through the NMDA-receptor-activated ion channels. L-Glutamate, a mixed agonist acting at NMDA receptors^{2,6,7}, also evoked Mg^{2+} -sensitive increases in $[Ca^{2+}]_i$. Figure 2b shows the responses to a constant dose of NMDA recorded at two membrane potentials; the inward current activated by NMDA at -60 mV was associated with an increase in $[Ca^{2+}]_i$, whereas the outward current activated by NMDA at $+60$ mV did not increase $[Ca^{2+}]_i$. In Mg^{2+} -free medium the NMDA current-voltage relationship was linear at membrane potentials more positive than -60 mV^{4,5,7}, and in the present experiments the increase in $[Ca^{2+}]_i$ evoked by constant doses of NMDA varied with the driving force for inward current over the membrane potential range 0 to -60 mV, suggesting that inward flux of Na^+ or Ca^{2+} through the ion channels opened by NMDA causes the increase in $[Ca^{2+}]_i$. At membrane potentials positive to $+10$ mV, NMDA did not induce increases in $[Ca^{2+}]_i$ detectable with arsenazo III. These experiments indicate that receptor activation *per se* is insufficient to account for the increase in $[Ca^{2+}]_i$ described here, and suggest that under

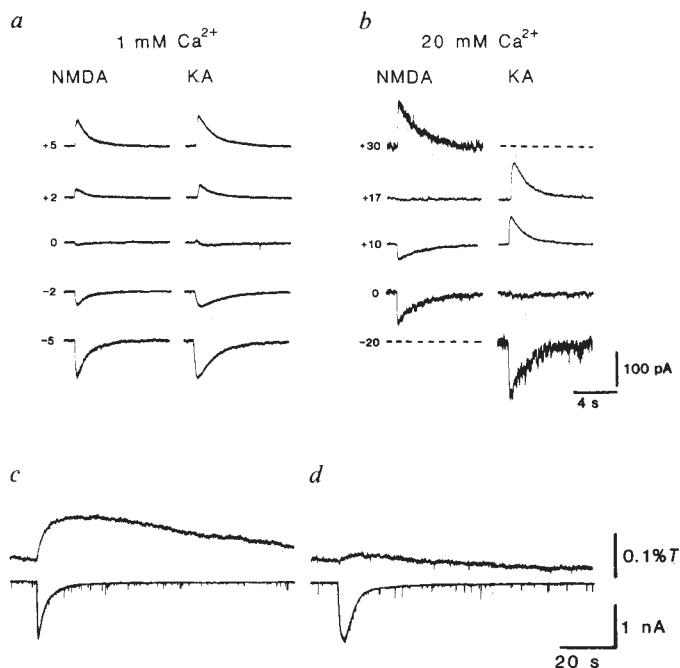


Fig. 3 Measurements of reversal potential suggest that NMDA channels are more permeable to Ca^{2+} than are KA channels. **a**, Current responses of one spinal cord neurone bathed in medium containing 1 mM Ca^{2+} , to pressure application of NMDA (60 ms, 1 mM) and KA (200 ms, 300 μ M) as the membrane potential (under voltage clamp) was varied over the range -5 to $+5$ mV; the reversal potential of the response to both excitatory amino acids is close to 0 mV. **b**, Current responses of a second spinal cord neurone, bathed in medium containing 20 mM Ca^{2+} , to application of NMDA (90 ms, 1 mM) and KA (120 ms, 300 μ M) as the membrane potential was varied over the range -20 to $+30$ mV. The reversal potential to NMDA is shifted to $+17$ mV, whereas reversal of the response to KA occurs at 0 mV. Assuming equal permeability (P) of monovalent cations through NMDA receptor channels, this result suggests that P_{Ca}/P_{Na} is ~ 5 , which compares with a value of 0.22 for nicotinic acetylcholine receptor channels^{33,34}. **c**, **d**, Optical measurements of $[Ca^{2+}]_i$ transients recorded from one spinal cord neurone bathed in medium containing 2.5 mM Ca^{2+} evoked by pressure application of NMDA (**c**: 200 ms, 1 mM) and KA (**d**: 70 ms, 100 μ M); the responses were matched for similar peak inward current activation by each of the excitatory amino acids at a holding potential of -60 mV. Note that the increase in $[Ca^{2+}]_i$ evoked by NMDA is much greater than that evoked by KA, a result compatible with the interpretation of the measurements of reversal potential shown in **a** and **b**.

our experimental conditions the production of $InsP_3$ does not play a major part in the response to excitatory amino acids. Our results do not by themselves exclude the possibility that Ca^{2+} or Na^+ influx through ion channels activated by NMDA triggers the intracellular release of Ca^{2+} , but the results presented below suggest that NMDA channels are indeed permeable to Ca^{2+} .

There is good evidence that the unitary properties of the ion channels linked to NMDA receptors are different from those linked to other excitatory amino-acid receptors in the vertebrate CNS^{16,24}. To determine whether these ion channels show differences in their permeability to Ca^{2+} , we measured the reversal potential of responses to NMDA and KA with extracellular calcium concentrations ($[Ca^{2+}]_o$) of 1 and 20 mM (Fig. 3a, b). A depolarizing shift of the NMDA reversal potential on increasing the $[Ca^{2+}]_o$ provides direct evidence that NMDA channels have a measurable calcium permeability. In contrast, the reversal potential of responses evoked by KA showed little Ca^{2+} dependence. Recently, Ca^{2+} dependence of the NMDA reversal potential²⁵ has been confirmed using single-channel recording experiments²⁶. Arsenazo III measurements also

differed between NMDA and KA (Fig. 3c, d), and in individual spinal cord neurones KA-evoked increases in $[Ca^{2+}]_i$ were always much smaller than those evoked by NMDA. These experiments suggest that Na^+ is a poor trigger for inducing an increase in $[Ca^{2+}]_i$, since in several neurones the inward (Na^+) current activated by KA produced no detectable arsenazo III signal. However, our results do not exclude the possibility that Ca^{2+} influx through ion channels activated by NMDA triggers release of Ca^{2+} from intracellular stores²⁷, contributing further to the NMDA-evoked arsenazo III signals reported here. Although the present results suggest a high Ca^{2+} permeability of NMDA-receptor-activated channels (Fig. 3), the net flux of monovalent cations (that is, conductance) decreases in the presence of Ca^{2+} . This reflects interactions between permeant ions within the channel with Ca^{2+} acting as both a permeant ion and as a blocker of monovalent cation flux^{25,26,28}.

The experiments reported here provide evidence for an agonist-triggered increase in $[Ca^{2+}]_i$ in mammalian spinal cord neurones. Previously, ion-sensitive microelectrodes were used to measure changes in intracellular ionic activity triggered by excitatory amino acids in frog motoneurons⁹. The latter experiments suggested an increase in both $[Na^+]_i$ and $[Ca^{2+}]_i$ during perfusion with L-glutamate but the results were difficult to interpret clearly as (1) neurones were not voltage-clamped and thus it is difficult to separate the relative contributions of Ca^{2+} influx via voltage-dependent calcium channels and agonist-activated channels, and (2) L-glutamate is a mixed agonist that acts at multiple subtypes of excitatory amino-acid receptor^{2,6,7}.

The response to NMDA-receptor activation thus provides a second source of calcium flux, distinct from that resulting from conventional voltage-dependent calcium channels, which may have important long-term effects on excitability. Our finding that the ion channels linked to the NMDA receptor subtype are more permeable to Ca^{2+} than those linked to KA receptors, has implications for the role of excitatory amino-acid receptors in CNS function. It is possible that Ca^{2+} influx activated by NMDA receptors underlies the synaptic plasticity generating long-term potentiation, as the latter is prevented by intracellular injection of EGTA to chelate Ca^{2+} (ref. 29), or by blocking NMDA receptors with selective antagonists³⁰. For example, Ca^{2+} influx localized at transmitter-operated ion channels could have a role in organizing and regulating postsynaptic structures in an appropriate spatial relation to transmitter-releasing presynaptic terminal boutons, and it is important to consider that Ca^{2+} influx occurring at NMDA receptors located on dendritic spines might produce an especially large but localized elevation in intracellular Ca^{2+} concentration, due to restriction of Ca^{2+} diffusion along the narrow shaft of the spine. In addition, our results have some bearing on the mechanisms of desensitization of NMDA receptors, as the link that has been demonstrated between $[Ca^{2+}]_i$ and desensitization of nicotinic receptors at the neuromuscular junction^{31,32} may occur also for other receptor-ionophore complexes. Thus our results may help to explain the similar desensitization evoked by either large doses of NMDA or depolarizing voltage jumps⁷, which trigger Ca^{2+} entry through NMDA channels and voltage-dependent calcium channels, respectively.

Received 3 January; accepted 1 April 1986.

- Krogsgaard-Larsen, P., Honoré, T., Hansen, J. J., Curtis, D. R. & Lodge, D. *Nature* **284**, 64–66 (1980).
- Watkins, J. C. & Evans, R. H. *A. Rev. Pharmac. Tox.* **21**, 165–205 (1981).
- McLennan, H. *Prog. Neurobiol.* **20**, 251–271 (1983).
- Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. & Prochiantz, A. *Nature* **307**, 462–465 (1984).
- Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. *Nature* **309**, 261–263 (1984).
- Mayer, M. L. & Westbrook, G. L. *J. Physiol., Lond.* **354**, 29–53 (1984).
- Mayer, M. L. & Westbrook, G. L. *J. Physiol., Lond.* **361**, 65–90 (1985).
- Dingledine, R. *J. Physiol., Lond.* **343**, 385–405 (1983).
- Bührle, C. P. & Sonnhof, U. *Pflügers Arch. ges. Physiol.* **396**, 154–162 (1983).
- Zanotto, L. & Heinemann, U. *Neurosci. Lett.* **35**, 79–84 (1983).
- Pumain, R. & Heinemann, U. *J. Neurophysiol.* **53**, 1–16 (1985).
- Lansman, J. B., Hess, P. & Tsien, R. W. *J. gen. Physiol.* (in the press).

- Ault, B., Evans, R. H., Francis, A. S., Oakes, D. J. & Watkins, J. C. *J. Physiol., Lond.* **307**, 413–428 (1980).
- Crunelli, V. & Mayer, M. L. *Brain Res.* **311**, 392–396 (1984).
- Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. *Pflügers Arch. ges. Physiol.* **391**, 85–100 (1981).
- Cull-Candy, S. G. & Ogden, D. C. *Proc. R. Soc. B224*, 367–373 (1985).
- Hagiwara, S. & Bylerly, L. A. *Rev. Neurosci.* **4**, 69–125 (1981).
- Smith, S. J., MacDermott, A. B. & Weight, F. F. *Nature* **304**, 350–352 (1983).
- Gorman, A. L. F. & Thomas, M. V. *J. Physiol., Lond.* **308**, 259–285 (1980).
- Berridge, M. J. & Irvine, R. F. *Nature* **312**, 315–321 (1984).
- Sladeczek, F., Pin, J. P., Récasens, M., Bockaert, J. & Weiss, S. *Nature* **317**, 717–719 (1985).
- Schoffeleer, A. M. N. & Mulder, A. H. *J. Neurochem.* **40**, 615–621 (1983).
- Evans, R. H. & Watkins, J. C. *J. Physiol., Lond.* **277**, 57P (1977).
- Nowak, L. M. & Ascher, P. *Soc. Neurosci. Abstr.* **10**, 23 (1984).
- Mayer, M. L. & Westbrook, G. L. *Soc. Neurosci. Abstr.* **11**, 785 (1985).
- Ascher, P. & Nowak, L. *J. Physiol., Lond. Proc.* (in the press).
- Fabiato, A. & Fabiato, F. *Ann. N.Y. Acad. Sci.* **307**, 491–522 (1978).
- Nowak, L. M. & Ascher, P. *Soc. Neurosci. Abstr.* **11**, 953 (1985).
- Lynch, G., Larson, J., Kelso, S., Barrinuevo, G. & Schottler, F. *Nature* **305**, 719–721 (1983).
- Collingridge, G. L., Kehl, S. J. & McLennan, H. *J. Physiol., Lond.* **334**, 33–46 (1983).
- Parsons, R. L. in *Calcium in Drug Action* (ed. Weiss, G. B.) 289–314 (Plenum, New York, 1978).
- Miledi, R. *Proc. R. Soc. B209*, 447–452 (1980).
- Adams, D. J., Dwyer, T. M. & Hille, B. *J. gen. Physiol.* **75**, 493–510 (1980).
- Edwards, C. *Neuroscience* **7**, 1335–1366 (1982).

Replacing the complementarity-determining regions in a human antibody with those from a mouse

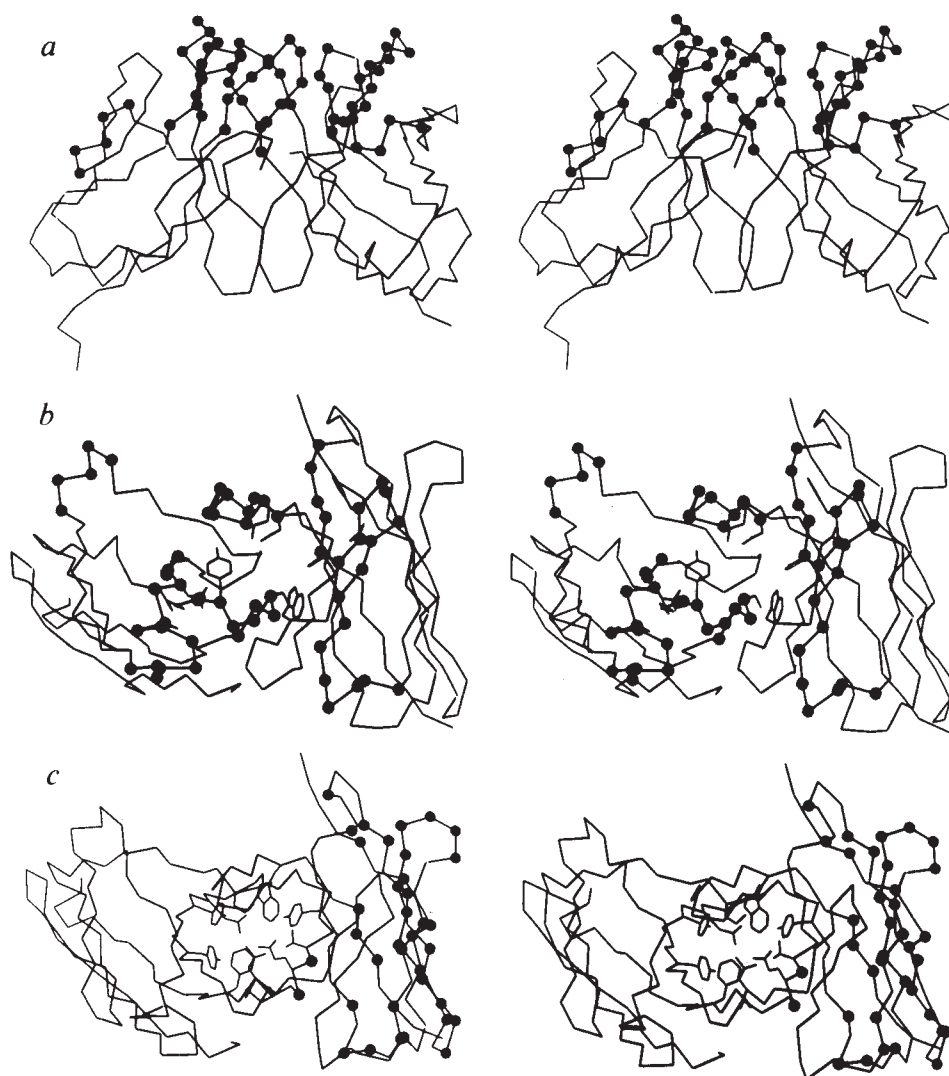
Peter T. Jones, Paul H. Dear, Jefferson Foote, Michael S. Neuberger & Greg Winter

Laboratory of Molecular Biology, Medical Research Council, Hills Road, Cambridge CB2 2QH, UK

The variable domains of an antibody consist of a β -sheet framework with hypervariable regions (or complementarity-determining regions—CDRs) which fashion the antigen-binding site. Here we attempted to determine whether the antigen-binding site could be transplanted from one framework to another by grafting the CDRs. We substituted the CDRs from the heavy-chain variable region of mouse antibody B1–8, which binds the hapten NP-cap (4-hydroxy-3-nitrophenacetyl caproic acid; $K_{NP-cap} = 1.2 \mu M$), for the corresponding CDRs of a human myeloma protein. We report that in combination with the B1–8 mouse light chain, the new antibody has acquired the hapten affinity of the B1–8 antibody ($K_{NP-cap} = 1.9 \mu M$). Such 'CDR replacement' may offer a means of constructing human monoclonal antibodies from the corresponding mouse monoclonal antibodies.

The three-dimensional structures of several immunoglobulins show that the variable domains consist of two β -sheets pinned together by a disulphide bridge, with their hydrophobic faces packed together^{1–3}. The individual β -strands are linked by loops which at one tip of the β -sheet may fashion a binding pocket for small haptens^{1,2}. Sequence comparisons among heavy- and light-chain variable domains (V_H and V_L respectively) reveal that each domain has three CDRs flanked by four relatively conserved regions (framework regions—FRs)⁴. As seen in the structure of the human myeloma protein NEWM (Fig. 1), the CDRs include each of the three main loops. Often the CDRs also include the ends of the β -strands, suggesting that side chains at the ends of the β -strands may help to fix the conformation or orientation of the loops. The framework regions form the bulk of the β -sheet, although for example in the V_H domain of NEWM, FR1 includes part of the loop between the two β -sheets and CDR2 not only forms a loop but a complete β -strand (Fig. 1). The structure of the β -sheet framework is similar in different antibodies, as the packing of different side chains is accommodated by slight shifts between the two β -strands⁵. Furthermore, the packing together of V_L and V_H FRs is conserved⁶, therefore the orientation of V_L with respect to V_H is fixed. We wondered whether the FRs represent a simple β -sheet scaffold on which new binding sites may be built, and

Fig. 1 Stereo pairs of the V_H (right) and V_L (left) domains of the human myeloma protein NEWM^{1,8} generated using the computer graphics program FRODO²⁵. The tracings indicate the backbone of C^α atoms for the framework regions. *a*, The C^α atoms of the CDRs (●) cluster at the tip of the variable domain. *b*, A view into the hapten binding pocket with the CDRs in the order (clockwise from noon): V_H CDR3, CDR1 and CDR2, and V_L CDR3, CDR1 and CDR2. The side chains lining the binding pocket (V_L A 28, N 30, Y 90, S 93, R 95; V_H W 47, Y 50, F 52, I 100, A 101) lie almost entirely in the CDRs. *c*, The C^α atoms in the NEWM V_H domain are marked (●) where side chains in the mouse B1-8 V_H domain are different. The side chains (V_L Y 35, Q 37, A 42, P 43, Y 86, F 99; V_H V 37, Q 39, L 45, Y 94, W 107) involved in packing V_H and V_L framework regions are traced. In the V_H domain all these side chains are conserved in mouse B1-8.



whether the structure of the CDRs (and antigen binding) is therefore independent of the FR context. To answer these questions experimentally, we have grafted the CDRs from one antibody to another, to determine whether antigen binding transfers with the CDRs.

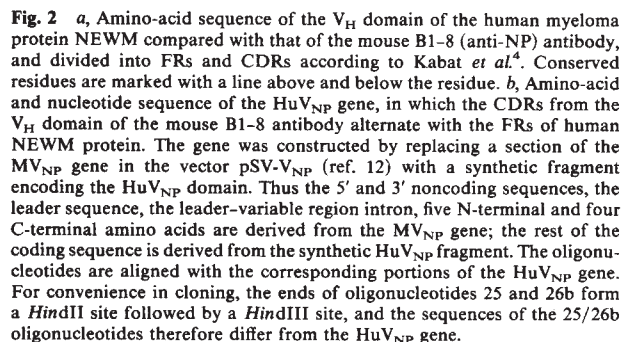
We grafted the CDRs from the V_H domain of the mouse monoclonal antibody B1-8 (ref. 7) into the V_H domain of the human myeloma protein NEWM, whose crystallographic structure is known^{1,8}. The V_H domain of the CDR donor (B1-8) is attached to a μ constant region and associated with a mouse λ 1 light chain, and the antibody is directed against the hapten NP-cap. Both the V_H and V_L domains seem to have a role in determining the affinity of the antibody for NP-cap as the substitution of either domain by other, often highly related variable domains can destroy hapten binding (refs 7, 9 and M.S.N., unpublished results). In the V_H domain, each of the CDRs has been implicated in NP-cap binding¹⁰, but the class of constant domains attached to V_H does not seem to affect binding of hapten^{11,12}. The CDRs from the V_H domain of antibody B1-8 (ref. 13) are longer than the CDRs which they replace in NEWM⁴ and this may give rise to a deeper binding pocket.

Most of the residues conserved between the V_H domains of B1-8 and NEWM are located in FR2, FR4 and the carboxy-terminal third of FR3 (Fig. 2a) and largely form the region of β -sheet which is packed against the light chain. Therefore, it might be expected that the V_H domain of B1-8 (hereafter abbreviated to MV_{NP}) and the hybrid B1-8/NEWM domain (HuV_{NP}) would dock in a similar manner with the mouse V_L domain to form the antigen-combining site⁶. The more variable

FR1 and N-terminal two-thirds of FR3 form the other β -sheet which is exposed to solvent (Fig. 1c).

The gene encoding the HuV_{NP} domain was constructed by gene synthesis (Fig. 2b). We then constructed a plasmid, pSV- $HuV_{NP}H\epsilon$, in which the HuV_{NP} domain is linked to a human ϵ constant region, and cloned into a pSV2gpt-derived vector¹⁴. The plasmid DNA was introduced into cells of the J558L mouse myeloma by spheroplast fusion. J558L secretes λ 1 light chains which have been shown to associate with heavy chains containing a MV_{NP} variable domain, to create a binding site for NP-cap or the related hapten NIP-cap (3-iodo-4-hydroxy-5-nitrophenyl-acetyl caproic acid)⁷. As the plasmid pSV- $HuV_{NP}H\epsilon$ contains the *gpt* marker (encoding guanine phosphoribosyltransferase), stably transfected myeloma cells could be selected in medium containing mycophenolic acid¹⁴; transfectants would be expected to secrete an antibody (HuV_{NP} -IgE) with a heavy chain composed of a HuV_{NP} variable domain and human ϵ constant regions, and the λ 1 light chain of the J558L myeloma. The culture supernatants of several *gpt*⁺ clones were assayed by radioimmunoassay and found to contain NIP-cap-binding antibody. The antibody secreted by one such clone was purified from the culture supernatant by affinity chromatography on NIP-cap-Sepharose, and by SDS-polyacrylamide gel electrophoresis the protein was indistinguishable from the mouse chimaeric MV_{NP} -IgE (ref. 12) (results not shown). The HuV_{NP} -IgE antibody competes effectively with MV_{NP} -IgE for binding to both anti-human ϵ (Fig. 3a) and NIP-cap coupled to bovine serum albumin (NIP-BSA) (Fig. 3b).

The affinities of HuV_{NP} -IgE for NP-cap and NIP-cap were



Methods. The synthetic gene for HuV_{NP} was constructed as a *Pst*I-*Hind*III fragment. The nucleotide sequence was derived from the protein sequence using the computer program ANALYSEQ²⁶ with optimal codon usage taken from the sequences of mouse constant-region genes. The oligonucleotides used in synthesis (1-26b, 28 in total) vary in size from 14 to 59 bases and were made on a Biosearch SAM or an Applied Biosystems machine, and purified on 8 M urea/polyacrylamide gels²⁷. The oligonucleotides were assembled in eight single-stranded blocks (A-D and A'-D') containing oligonucleotides 1, 3, 5 and 7 (block A), 2, 4, 6 and 8 (block A'), 9, 11, 13a and 13b (block B), 10a, 10b and 12/14 (block B'), 15 and 17 (block C), 16 and 18 (block C'), 19, 21, 23 and 25 (block D), and 20, 22/24, 26a and 26b (block D'). In a typical assembly, for example of block A, 50 pmol of oligonucleotides 1, 3, 5 and 7 were phosphorylated at the 5' end with T₄ polynucleotide kinase and mixed with 5 pmol of the terminal oligonucleotide which had been phosphorylated with 5 μ Ci of [γ -³²P]ATP (Amersham; 3,000 Ci mmol⁻¹). These oligonucleotides were annealed by heating to 80 °C and cooling to room temperature over 30 min with unkinased oligonucleotides 2, 4 and 6 as splints in 150 μ l of 50 mM Tris-HCl pH 7.5, 10 mM MgCl₂. For the ligation, ATP (1 mM) and dithiothreitol (10 mM) were added, together with 50 units of T₄ DNA ligase (Anglian Biotechnology Ltd), and the mixture was incubated for 30 min at room temperature. EDTA was added to 10 mM, the sample extracted with phenol, precipitated from ethanol, dissolved in 20 μ l of water and boiled for 1 min with an equal volume of formamide dyes. Then the sample was loaded onto a thin (0.3 mm) 8 M urea/10% polyacrylamide gel²⁷ and a band of the expected size detected by autoradiography and eluted by soaking. The two full-length single strands were assembled from A-D and A'-D' using splint oligonucleotides; thus, A-D were annealed and ligated in 30 μ l as above with 100 pmol each of oligonucleotides 10a, 16 and 20 as splints, then incubated overnight (A'-D' were constructed with oligonucleotides 7, 13b and 17 as splints). After phenol/ether extraction blocks A-D were annealed with blocks A'-D', small amounts were cloned in the vector M13 mp18 (ref. 28) then cut with *Pst*I and *Hind*III, and the gene sequenced by the dideoxy technique²⁹. The MV_{NP} gene was transferred as a *Hind*III-*Bam*HI fragment from the vector pSV-V_{NP} (ref. 12) to the vector M13mp8 (ref. 30). To facilitate the replacement of MV_{NP} coding sequences by the synthetic HuV_{NP} fragment, three *Hind*II sites were removed from the 5' noncoding sequence by site-directed mutagenesis, and a new *Hind*II site subsequently introduced near the end of FR4. By cutting the vector with *Pst*I and *Hind*II, most of the V_{NP} coding sequence falls out and the synthetic fragment could be introduced as a *Pst*I-*Hind*II fragment. The sequence at the *Hind*II site was corrected to give NEWM FR4 by site-directed mutagenesis. The *Hind*III-*Bam*HI fragment, now carrying the HuV_{NP} gene, was excised from M13 and cloned back into pSV-V_{NP} to replace the MV_{NP} gene (and yield the vector pSV-HuV_{NP}). Finally, the heavy-chain constant domains of human IgE (ref. 31) were introduced as a *Bam*HI fragment to yield the vector pSV-HuV_{NP}H_{ec}, which was transfected into the myeloma line J558J by spheroplast fusion. The sequence of the HuV_{NP} gene in pSV-HuV_{NP}H_{ec} was checked by re-cloning the *Hind*III-*Bam*HI fragment back into M13mp8 (ref. 30).

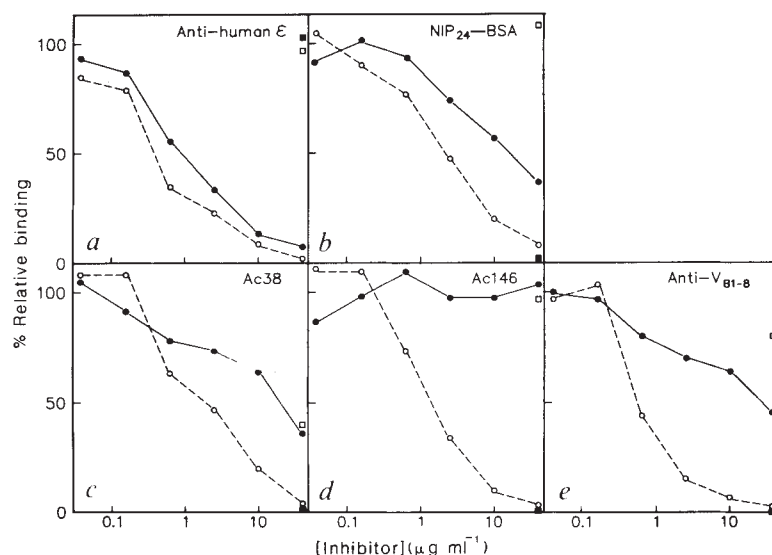
	K_{NP-cap} (μM)	$K_{NIP-cap}$ (μM)
MV _{NP} -IgE	1.2 ± 0.1	0.02 ± 0.01
HuV _{NP} -IgE	1.9 ± 0.2	0.07 ± 0.02

The affinity of HuV_{NP}-IgE and MV_{NP}-IgE for NP-cap was determined by fluorescence quenching with excitation at 295 nm and emission observed at 340 nm (ref. 22). Antibody solutions were diluted to 100 nM in phosphate-buffered saline, filtered (0.45 μ m-pore cellulose acetate) and titrated with NP-cap in the range 0.2–20 μ M. As a control, the mouse D1-3 antibody²³, which does not bind hapten, was titrated in parallel. A decrease in the ratio of the fluorescence of HuV_{NP}-IgE or MV_{NP}-IgE (as appropriate) to that of the D1-3 antibody was taken as being proportional to NP-cap occupancy of the antigen-binding sites. The maximum quench was ~40% for both HuV_{NP}-IgE and MV_{NP}-IgE, and hapten dissociation constants were determined from least-squares fits of triplicate data sets to a hyperbola. The concentration of NP-cap was varied from 10 to 300 nM, and ~50% quenching of fluorescence was observed at saturation. As the antibody concentrations were comparable to the values of the dissociation constants, data were fitted by least-squares to an equation²⁴ describing tight binding inhibition.

then measured directly using the fluorescence quench technique, and compared with those of MV_{NP}-IgE (Table 1). The antibodies HuV_{NP}-IgE and MV_{NP}-IgE have similar affinities for either hapten (NP-cap or NIP-cap), and although the affinity of HuV_{NP}-IgE for both haptens is slightly lower than that of MV_{NP}-IgE (2–3-fold, 0.3–0.6 kcal mol⁻¹), the difference in affinity is less than expected for loss of either a hydrogen bond or van der Waals' contact from the active site of an enzyme^{15,16}. Thus, it seems that binding affinity and specificity for hapten can be conferred on a human antibody by grafting in the CDRs from an appropriate mouse antibody.

Is this result likely to be general? This would assume (1) that antigen usually binds to the CDRs, and any contacts to the FRs are made to the polypeptide backbone or to conserved side chains, and (2) that substitutions in the FRs do not usually affect the conformation of the CDR loops. These assumptions seem reasonable: thus, in the structure of a complex of the D1-3 antibody with lysozyme (R. A. Mariuzza, S. Phillips and R. J. Poljak, personal communication) most contacts to the lysozyme are made by the CDRs, but there is also a hydrogen bond in FR1 of the V_H domain from the β -OH of Thr 30 (often conserved or replaced by Ser). Similarly, the conformation of CDR loops

Fig. 3 Comparison of HuV_{NP} and MV_{NP} IgEs in binding inhibition assays. Various concentrations of HuV_{NP}-IgE (●) and MV_{NP}-IgE (○) were used to compete the binding of radiolabelled MV_{NP}-IgE to polyvinyl microtitre plates that had been coated with *a*, sheep anti-human ϵ antiserum (Seward Laboratory); *b*, (NIP-cap)₂₄-BSA; *c*, Ac38 anti-idiotypic antibody; *d*, Ac146 anti-idiotypic antibody; *e*, rabbit anti-MV_{NP} antiserum. Binding was also carried out in the presence of MV_{NP}-IgM antibody JW1/2/2 (ref. 32) (■) as well as in the presence of JW5/1/2 (□), which is an IgM antibody that differs from JW1/2/2 at 13 residues mainly located in V_H CDR2 (M.S.N., unpublished results). Values of binding are relative to the binding in the absence of inhibitor.



between β -strands depends on loop size and specific interactions of the loop back to the β -sheet. However, in the same class of variable domains (V_H, V_K or V_L) these interactions are usually conserved (ref. 5 and A. M. Lesk and C. Chothia, personal communication).

While human monoclonal antibodies have therapeutic potential in human disease, they can be difficult to prepare¹⁷ and treatment of patients with mouse monoclonal antibodies often increases the titre of circulating antibody against the mouse immunoglobulin¹⁸. As chimaeric antibodies containing human constant domains^{12,19,20} and variable domains made by grafting mouse CDRs into human FRs, could have therapeutic potential, we wondered whether the HuV_{NP}-IgE antibody loses antigenic determinants associated with the MV_{NP} variable region (idiotopes). The binding of HuV_{NP}-IgE and MV_{NP}-IgE to both monoclonal and polyclonal anti-idiotypic antibodies directed against the MV_{NP} domain was examined by using inhibition assays. As shown in Fig. 3*d*, the HuV_{NP}-IgE antibody has lost the MV_{NP} idiotype determinant recognized by antibody Ac146 (ref. 21). Furthermore, HuV_{NP}-IgE also binds the antibody Ac38 (ref. 21) less well (Fig. 3*c*), therefore it is not surprising that HuV_{NP}-IgE has lost many of the determinants recognized by a polyclonal rabbit anti-idiotypic antiserum (Fig. 3*e*). While the loss of idiotype determinants that accompanies 'humanizing' of the V_H region is reassuring in view of potential therapeutic applications, it does suggest that the recognition of the hapten and of anti-idiotypic antibodies is not equivalent. Thus the HuV_{NP}-IgE antibody retains hapten binding but has lost idiotype determinants, indicating that the immunoglobulin uses different sites to bind hapten and anti-idiotypic antibodies. It appears, therefore, that both FR and CDR side chains form the binding site for these anti-idiotypes, but mainly CDR side chains interact with hapten.

We thank C. Milstein for suggesting this project, K. Rajewsky and M. Reth for the anti-idiotypic antibodies Ac38 and Ac146, and A. M. Lesk, C. Chothia, R. J. Leatherbarrow and C. Milstein for helpful discussions. J.F. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

10. Reth, M., Bothwell, A. L. M. & Rajewsky, K. in *Immunoglobulin Idiotypes and Their Expression* (eds Janeway, C., Wiggall, H. & Fox, C. F.) 169-178 (Academic, New York, 1981).
11. Neuberger, M. S. & Rajewsky, K. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1138-1142 (1981).
12. Neuberger, M. S. *et al. Nature* **314**, 268-270 (1985).
13. Bothwell, A. L. M. *et al. Cell* **24**, 625-637 (1981).
14. Mulligan, R. C. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2072-2076 (1983).
15. Fersht, A. R. *et al. Nature* **314**, 235-238 (1985).
16. Fersht, A. R., Wilkinson, A. J., Carter, P. & Winter, G. *Biochemistry* **24**, 5858-5861 (1985).
17. Boyd, J. E., James, K. & McClelland, D. B. L. *Trends Biotechnol.* **2**, 70-77 (1984).
18. Shawler, D. L., Bartholomew, R. M., Smith, L. M. & Dilmann, R. O. *J. Immun.* **135**, 1530-1535 (1985).
19. Morrison, S. L., Johnson, M. J., Herzenberg, L. A. & Oi, V. T. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6851-6855 (1984).
20. Boulianne, G. L., Hozumi, N. & Shulman, M. J. *Nature* **312**, 643-646 (1984).
21. Reth, M., Imanishi-Kari, T. & Rajewsky, K. *Eur. J. Immun.* **9**, 1004-1013 (1979).
22. Eisen, H. N. *Meth. med. Res.* **10**, 115-121 (1964).
23. Mariuzza, R. A. *et al. J. molec. Biol.* **170**, 1055-1058 (1983).
24. Segal, I. H. in *Enzyme Kinetics*, 73-74 (Wiley, New York, 1975).
25. Jones, T. A. in *Computational Crystallography* (ed. Sayre, D.) 303-310 (Clarendon, Oxford, 1982).
26. Staden, R. *Nucleic Acids Res.* **12**, 521-538 (1984).
27. Sanger, F. & Coulson, A. *FEBS Lett.* **87**, 107-110 (1978).
28. Yanisch-Perron, C., Vieira, J. & Messing, J. *Gene* **33**, 103-119 (1985).
29. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
30. Messing, J. & Vieira, J. *Gene* **19**, 269-276 (1982).
31. Flanagan, J. G. & Rabbitts, T. H. *EMBO J.* **1**, 655-660 (1982).
32. Neuberger, M. S., Williams, G. T. & Fox, R. O. *Nature* **312**, 604-608 (1984).

Regulation of human insulin gene expression in transgenic mice

Richard F Selden*, Marek J. Skośkiewicz†, Kathleen Burke Howie*, Paul S. Russell† & Howard M. Goodman*

Departments of *Molecular Biology and †Surgery, Massachusetts General Hospital, and Departments of *Genetics and †Surgery, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114, USA

Insulin is a polypeptide hormone of major physiological importance in the regulation of fuel homeostasis in animals (reviewed in refs 1, 2). It is synthesized by the β -cells of pancreatic islets, and circulating insulin levels are regulated by several small molecules, notably glucose, amino acids, fatty acids and certain pharmacological agents. Insulin consists of two polypeptide chains (A and B, linked by disulphide bonds) that are derived from the proteolytic cleavage of proinsulin, generating equimolar amounts of the mature insulin and a connecting peptide (C-peptide). Humans, like most vertebrates, contain one proinsulin gene^{3,4}, although several species, including mice⁵ and rats^{6,7}, have two highly homologous insulin genes. We have studied the regulation of serum insulin

Received 17 February; accepted 17 March 1986.

1. Poljak, R. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 3305-3310 (1973).
2. Segal, D. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **71**, 4298-4302 (1974).
3. Marquart, M., Deisenhofer, J., Huber, R. & Palm, W. *J. molec. Biol.* **141**, 369-391 (1980).
4. Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M. & Perry, H. in *Sequences of Proteins of Immunological Interest* (U.S. Department of Health and Human Services, 1983).
5. Lesk, A. M. & Chothia, C. *J. molec. Biol.* **160**, 325-342 (1982).
6. Chothia, C., Novotny, J., Bruccoleri, R. & Karplus, M. *J. molec. Biol.* **186**, 651-663 (1985).
7. Reth, M., Hämmerling, G. J. & Rajewsky, K. *Eur. J. Immun.* **8**, 393-400 (1978).
8. Saul, F. A., Amzel, M. & Poljak, R. J. *J. biol. Chem.* **253**, 585-597 (1978).
9. Brüggemann, M., Radbruch, A. & Rajewsky, K. *EMBO J.* **1**, 629-634 (1982).

levels and of insulin gene expression by generating a series of transgenic mice containing the human insulin gene. We report here that the human insulin gene is expressed in a tissue-specific manner in the islets of these transgenic mice, and that serum human insulin levels are properly regulated by glucose, amino acids and tolbutamide, an oral hypoglycaemic agent.

The human insulin gene that we have used to generate transgenic mice is contained within a 12.5-kilobase (kb) *EcoRI* fragment that was isolated from a genomic library⁴. Of 46 mice born after one series of single-cell embryo microinjections, three contained human insulin gene sequences as detected by Southern hybridization analysis (Fig. 1a). A human C-peptide radioimmunoassay (Behringwerke) was used to monitor expression of the human insulin gene in the transgenic mice and their offspring. Several hundred transgenic and control mice have been analysed under a variety of physiological circumstances (see below), and the transgenic mice show variable levels of human C-peptide in their sera, whereas control mice show no such expression.

The tissue specificity of human insulin gene expression in these transgenic mice was examined by both RNA analyses and pancreatic islet function studies. Northern hybridizations using a human insulin complementary DNA probe demonstrated that total pancreatic RNA from both transgenic (Fig. 1b, lane 11) and control (lane 1) mice hybridized to the human insulin cDNA probe, whereas RNAs from transgenic spleen, kidney, brain, lung, liver, salivary gland, intestine, heart and muscle (lanes 2–10, respectively) showed no hybridization. Because no transgenic tissues other than pancreas were found to express detectable levels of insulin RNA, insulin expression in both transgenic and control pancreas was studied to determine whether the transgenic pancreas is a site of human insulin expression. Pancreatic islets from six transgenic and six control mice were isolated by collagenase digestion as described previously^{8,9} and cultured in groups of 80–100 islets per tissue culture well. The following day, aliquots of media were taken and human C-peptide levels measured. The samples from the transgenic islet wells contained 250–650 ng ml⁻¹ of human C-peptide, but the control islet wells contained no detectable human C-peptide. The cultured transgenic islets continued to express human C-peptide for several days. From these experiments, we conclude that the major site of human insulin expression in these transgenic mice is the endocrine pancreas.

Transgenic and control pancreas were stained with immunoperoxidase using a guinea pig anti-porcine insulin antibody and a goat anti-human C-peptide antibody (Fig. 2). The anti-porcine insulin antibody cross-reacted with both human and mouse insulin, and islets from both transgenic (Fig. 2a) and control (Fig. 2b) mice were stained. The size, distribution and number of islets were essentially the same in transgenic and control mice. The anti-human C-peptide antibody showed little or no cross-reactivity with mouse C-peptide, however, and the transgenic islets (Fig. 2c) were stained using this antibody whereas the control islets (Fig. 2d) were not. These immunohistochemistry data are consistent with the Northern analysis and islet function studies presented above, and demonstrate that the transgenic islets were specifically expressing human insulin.

Glucose and human C-peptide levels in the transgenic mice were studied under a variety of physiological conditions to determine whether normal glucose homeostasis was being preserved and whether expression of the human insulin gene was being regulated appropriately in these mice. Blood glucose regulation was studied by glucose tolerance tests. Transgenic offspring of mouse 16 and non-transgenic siblings were fasted overnight, given an intraperitoneal (i.p.) injection of glucose, and bled at various times after injection to determine serum glucose levels. The glucose tolerance curve from the transgenic mice was similar to that from the control mice (Fig. 3a). Of particular importance is the finding that the fasting and maximally stimulated glucose levels as well as the kinetics of

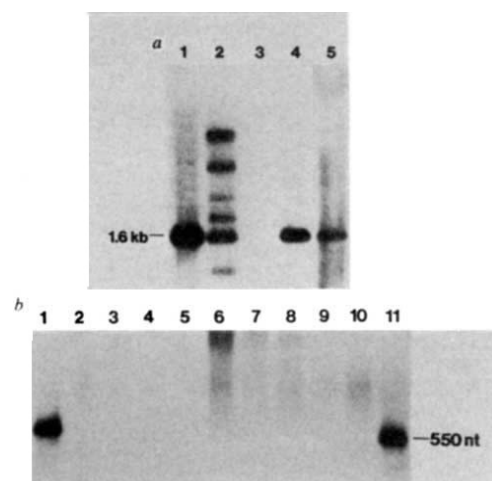


Fig. 1 Characterization of microinjected mice. **a**, Detection of human insulin sequences in total genomic DNA of microinjected mice. DNA samples digested with *PvuII* are from the following microinjected mice: lane 1, mouse 16, containing 5–10 copies of the human insulin gene fragment per haploid genome; lane 2, mouse 20, also containing 5–10 copies of the human insulin gene fragment per haploid genome; lane 3, mouse 21, negative for human insulin sequences, demonstrating that the endogenous mouse insulin genes do not hybridize significantly to the human insulin genomic probe; lane 4, human control, containing 1 copy of the human insulin gene per haploid genome; lane 5, mouse 38, containing 1–2 copies of the human insulin gene fragment per haploid genome (this DNA sample was analysed in a separate experiment in which the hybridizing band was of comparable intensity to the human control). DNA samples from each transgenic mouse contain a hybridizing band of ~1,600 bp which corresponds to a 1,594-bp *PvuII* fragment contained within the 12.5-kb human insulin DNA fragment⁴ used for microinjection. This result suggests that at least one structurally intact human insulin gene was incorporated into the genome of each transgenic animal, although the presence of multiple hybridizing bands in lane 2 indicates that other integration events are likely to have occurred during the embryogenesis of mouse 20. Mouse 16, a male, was bred to generate a colony of several hundred offspring, and the human insulin gene was transmitted in mendelian fashion, with ~50% of both the F₁ and F₂ offspring inheriting the injected DNA fragment. **b**, Detection of human and mouse insulin messenger RNAs in tissues of transgenic and control mice. Total cellular RNA samples are from transgenic and non-transgenic offspring of mouse 16 as follows: lane 1, non-transgenic pancreas; lane 2, transgenic spleen; lane 3, transgenic kidney; lane 4, transgenic brain; lane 5, transgenic lung; lane 6, transgenic liver; lane 7, transgenic salivary gland; lane 8, transgenic intestine; lane 9, transgenic heart; lane 10, transgenic muscle; lane 11, transgenic pancreas. Neither the mouse insulin cDNA nor gene sequences have been determined, but since the coding regions of the human and rat insulin mRNAs share 81% sequence homology¹⁵, it was expected that both transgenic and control pancreatic RNAs would hybridize to the human insulin cDNA probe.

Methods. A 12.5-kb *EcoRI* fragment containing the human insulin gene⁴ was isolated from pBR322 sequences by preparative gel electrophoresis and electroelution¹⁶. Fertilized mouse eggs for microinjection were recovered in cumulus from the oviducts of (C57×C3H)F₁ females that had mated with F₁ males several hours earlier. Approximately 1,000 copies of the human insulin gene fragment were microinjected into the male pronucleus of each fertilized egg. Microinjected eggs were implanted into the oviducts of 1-day pseudopregnant ICR foster mothers and carried to term¹⁷. Several weeks after the birth of animals that developed from microinjected eggs, total genomic DNA was prepared¹⁸ from mouse tails. For the Southern blot analysis in **a**, 8 µg of total genomic DNA for each mouse were digested with *PvuII*, subjected to electrophoresis on a 0.9% agarose gel and transferred to nitrocellulose¹⁹. The filter was then prehybridized overnight, hybridized to a ³²P-labelled genomic human insulin probe, washed and exposed to X-ray film. The genomic human insulin probe is a fragment that extends from a *BglII* site at -169 with respect to the transcriptional start site of the human insulin gene to an *AvaI* site at +644 (ref. 4). In addition to promoter sequences, this fragment contains the first intervening sequence, the first exon (including sequences encoding the signal peptide, B-peptide and a portion of C-peptide), and a portion of the second intervening sequence. Approximately 75% of the genomic human insulin probe consists of noncoding sequences that are not highly conserved between species, which explains its limited cross-hybridization with the endogenous mouse insulin genes (which can be detected only after long exposures). Total cellular RNA was isolated from tissue samples by the guanidinium isothiocyanate/caesium chloride technique²⁰. For Northern blot analysis, 4 µg of total RNA from each sample was subjected to electrophoresis on a 1.2% agarose-formaldehyde denaturing gel and transferred to nitrocellulose filters¹⁶. The filter was then prehybridized, hybridized to a ³²P-labelled human insulin cDNA¹⁵ probe, washed and exposed to X-ray film.

Fig. 2 Immunoperoxidase staining of transgenic and control pancreas. Pancreas samples were immersed in liquid nitrogen and 4- μ m tissue sections were prepared using a cryostat. Immunoperoxidase staining of serial sections was performed as described previously²¹, using either guinea pig anti-porcine insulin antibody (*a* and *b*) or goat anti-human C-peptide antibody (*c* and *d*). *a*, Transgenic pancreas stained with anti-insulin antibody (Arnel Products). *b*, Control pancreas stained with anti-insulin antibody. *c*, Transgenic pancreas stained with anti-human C-peptide antibody (Behringwerke). *d*, Control pancreas stained with anti-human C-peptide antibody (photographically enhanced to allow visualization of islets). Other transgenic tissues, including liver, adrenal and thyroid, are not stained using the guinea pig anti-porcine insulin antibody (not shown).

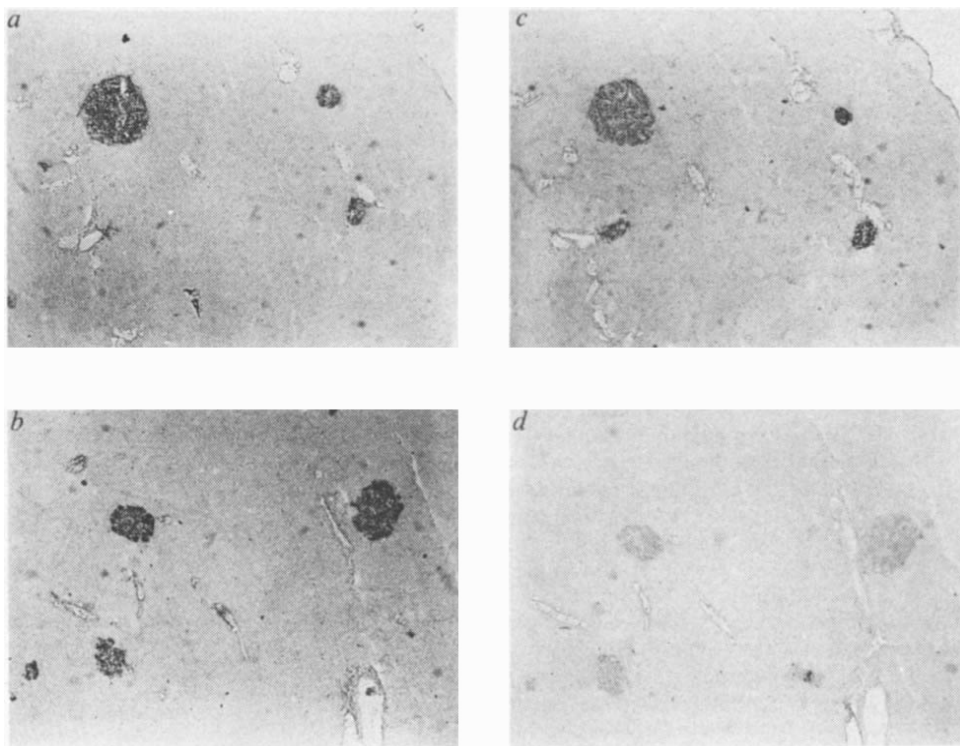
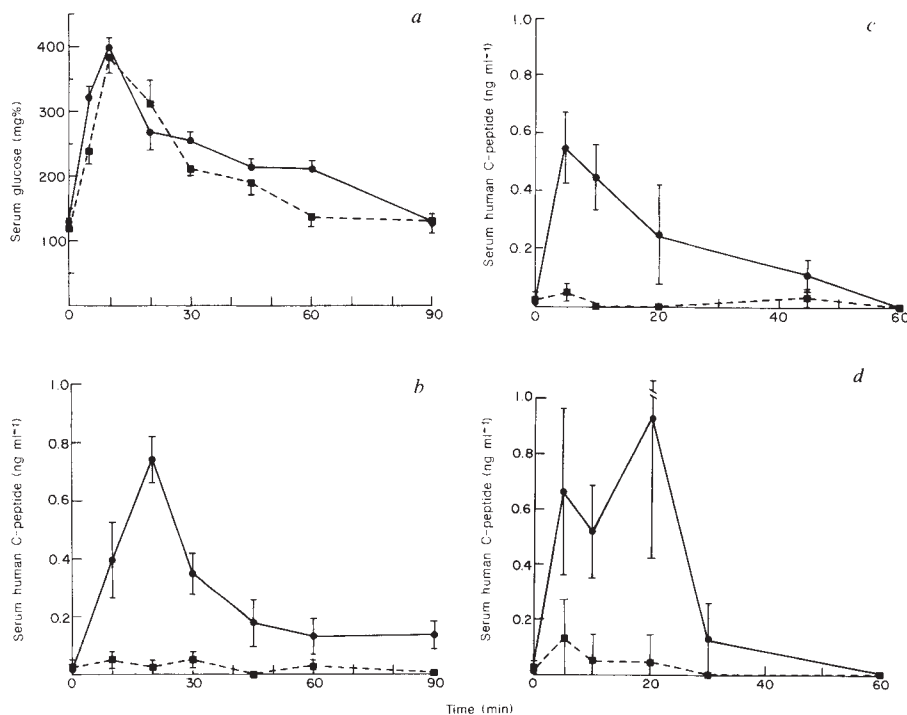


Fig. 3 *a*, Intraperitoneal glucose tolerance test.

Transgenic offspring of mouse 16 and non-transgenic siblings were fasted overnight, then given an i.p. injection of glucose (1 mg glucose per g body weight). Each mouse was then arbitrarily assigned to a group of four for serum sampling at 5, 10, 20, 30, 45, 60 or 90 min post-injection. Four mice did not receive glucose and were bled to determine fasting serum glucose levels. Each time point therefore represents an average of values from four animals, and standard deviation half-bars are indicated. Solid line, transgenic mice; dashed line, control mice. Serum glucose determinations were performed using a commercially available kit (Worthington) under the conditions recommended by the manufacturer. Intravenous and oral glucose tolerance tests performed as described above showed no statistically significant difference between the transgenic and control mice (data not shown). *b*, Human C-peptide levels during an i.p. glucose tolerance test. The experiment was performed as described in *a*, with each transgenic point representing an average of values from six animals (i.p. injection of 2 mg glucose per g body weight). Serum human C-peptide levels were measured using a commercially available kit (Behringwerke) under the conditions recommended by the manufacturer. Solid line, transgenic mice; dashed line, control mice. These data are similar to glucose tolerance results previously reported for both mice²² and humans². Preliminary experiments suggest that mouse 20 also expresses human C-peptide and responds to a glucose tolerance test, and more extensive studies will be performed when mouse 20 and mouse 38 have generated large colonies. An assay specific for mouse C-peptide is not available, and we were unable to compare levels of endogenous mouse C-peptide with levels of human C-peptide. *c*, Human C-peptide levels during an i.v. amino-acid tolerance test. Transgenic and control mice were fasted overnight, infused with a solution containing 0.5 mg arginine and 0.5 mg leucine, and bled at the indicated times for human C-peptide determination. Each time point represents the average of values from four animals. Solid line, transgenic mice; dashed line, control mice. *d*, Human C-peptide levels during an i.v. tolbutamide infusion test. Transgenic and control mice were fasted overnight, infused with a solution containing 0.5 mg of tolbutamide (Upjohn) and bled at the indicated times for human C-peptide determination. Each time point represents the average of values from two animals. Solid line, transgenic mice; dashed line, control mice.



the return to basal glucose levels were similar for the transgenic and control animals. In addition, intravenous (i.v.) administration of glucagon increased serum glucose levels by ~50% within 15 min in both transgenic and control mice. Taken together, these results strongly suggest that serum glucose levels were appropriately modulated in the transgenic mice. The weights of the transgenic mice, growth rates, feeding behaviour, reproductive capability and longevity appeared normal.

The role of human insulin in the regulation of blood glucose levels in transgenic mice was investigated by performing a glucose tolerance test on transgenic and control mice (Fig. 3b). No human C-peptide was detected in the sera of fasting transgenic mice, but within 10 min of i.p. administration of glucose, human C-peptide appeared in the serum, and peak levels were attained within ~20 min. By 45 min post-glucose, human C-peptide levels fell to values approaching the pre-stimulation or basal level. This pattern of human C-peptide expression correlates closely with the glucose tolerance curves presented above, and suggests that serum human insulin levels were being appropriately regulated by glucose. The control mice did not express any detectable human C-peptide, indicating that the human gene must have been the source of the human C-peptide in the transgenic animals.

Insulin is regulated by several other factors, including amino acids and certain pharmacological agents. An i.v. amino-acid infusion test was performed on fasting transgenic and control mice and human C-peptide levels in the serum were determined. Peak human C-peptide levels were seen within 5 min of amino-acid infusion and declined gradually over the next 40 min (Fig. 3c). Similarly, serum human C-peptide levels responded to tolbutamide, a sulphonylurea derivative known to promote insulin release¹⁰ (Fig. 3d). Within 20 min of i.v. tolbutamide administration, serum human C-peptide levels peaked, then decreased rapidly over the next 10 min. Tolbutamide has been used clinically to diagnose insulinomas¹¹ because in normal subjects serum insulin (or C-peptide) levels rapidly return to normal from their tolbutamide-induced peak, but in insulinoma patients elevated insulin levels persist. That the transgenic mice quickly regained basal serum human C-peptide levels supports the conclusion that their insulin expression was tightly regulated.

We have demonstrated that the human insulin gene is expressed in the pancreas of transgenic mice. Cell-type- and tissue-specific expression of human¹² and rat¹²⁻¹⁴ insulin genes has been documented in two other laboratories. A 230-base-pair (bp) region (from -103 bp to -333 bp with respect to the transcriptional start site) of the rat insulin I promoter was reported to be sufficient to allow tissue-specific expression of insulin/chloramphenicol acetyltransferase fusion genes in a hamster pancreatic cell line^{12,13}. Similarly, a rat insulin II/simian virus 40 large-T antigen fusion gene has been reported to cause the development of islet cell tumours in transgenic mice¹⁴. As both of these studies used fusion genes, the regulation of circulating human insulin could not be studied.

Serum insulin levels are regulated by glucose, amino acids, proteins and drugs such as the sulphonylurea derivatives. The human insulin gene in these transgenic mice is regulated appropriately by all of these agents, and serum glucose homeostasis is normal. These transgenic animals can therefore now be used to study several critical aspects of the physiological regulation of insulin gene expression, including the mechanisms controlling serum insulin and total β -cell insulin levels. Because at least one additional insulin gene is being expressed in the transgenic mice and total insulin RNA and protein levels are approximately the same as in control mice, the question of dosage compensation can be investigated. Moreover, our tolbutamide results indicate that drugs thought to affect human insulin metabolism can now be tested in an *in vivo* animal system. In a more general sense, the *in vivo* effects of various pharmacological agents on human gene expression and protein function can therefore be evaluated in a non-human setting.

Finally, it is noteworthy that a 12.5-kb DNA fragment contains sufficient information for the appropriate physiological regulation of insulin levels in these transgenic mice. The organism's ability to modulate foreign DNA sequences and proteins on a minute to minute basis clearly has important implications for both molecular biology and gene therapy.

We thank Dr Tom Wagner for instruction in microinjection, Victoria Roman for technical assistance and Patrick Mattoon for animal care. The human C-peptide assay was the gift of Dr H. H. Schoene and Behringwerke, AG. This work was supported by grants from Hoechst AG and the NIH (AM-07055).

Received 17 December 1985; accepted 18 March 1986.

- Steiner, D. F. & Tager, H. S. in *Endocrinology* Vol. 2 (eds DeGroot, L. J. et al.) 921-934 (Grune & Stratton, New York, 1979).
- Rubenstein, A. H. in *Endocrinology* Vol. 2 (eds DeGroot, L. J. et al.) 951-957 (Grune & Stratton, New York, 1979).
- Tager, H. et al. *Nature* **281**, 122-125 (1979).
- Bell, G. I. et al. *Nature* **284**, 26-32 (1980).
- Lally, P. A. & Chirgwin, J. M. *Cytogenet. cell. Genet.* **37**, 515 (1984).
- Cordell, B. et al. *Cell* **18**, 533-543 (1979).
- Lomedico, P. et al. *Cell* **18**, 545-558 (1979).
- Moskalewski, S. *Gen. comp. Endocr.* **5**, 342-353 (1965).
- Lacy, P. E. & Kostianovsky, M. *Diabetes* **16**, 35-39 (1967).
- Ganda, O. P. et al. *Diabetes* **24**, 354-361 (1975).
- Fajans, S. S. & Conn, J. W. *J. Lab. clin. Med.* **54**, 811 (1959).
- Walker, M. D., Edlund, T., Boulet, A. M. & Rutter, W. J. *Nature* **306**, 557-561 (1983).
- Edlund, T., Walker, M. D., Barr, P. J. & Rutter, W. J. *Science* **230**, 912-916 (1985).
- Hanahan, D. *Nature* **315**, 115-122 (1985).
- Bell, G. I. et al. *Nature* **282**, 525-527 (1979).
- Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
- Wagner, T. E. et al. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6376-6380 (1981).
- Rogol, A. D. et al. *Pediat. Res.* **19**, 489-492 (1985).
- Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18**, 5294-5299 (1979).
- Sobel, R. A., Blanchette, B. W., Bhan, A. B. & Colvin, R. B. *J. Immun.* **132**, 2402-2403 (1984).
- Larkins, R. G. *Diabetes* **22**, 251-255 (1973).

Genetic recombination between RNA components of a multipartite plant virus

Jozef J. Bujarski & Paul Kaesberg

Biophysics Laboratory and Department of Biochemistry,
University of Wisconsin-Madison, Madison, Wisconsin 53705, USA

Genetic recombination of DNA is one of the fundamental mechanisms underlying the evolution of DNA-based organisms and results in their diversity and adaptability. The importance of the role of recombination is far less evident for the RNA-based genomes that occur in most plant viruses and in many animal viruses. RNA recombination has been shown to promote the evolutionary variation of picornaviruses¹⁻⁴, it is involved in the creation of defective interfering (DI) RNAs of positive- and negative-strand viruses⁵⁻⁹ and is implicated in the synthesis of the messenger RNAs of influenza virus¹⁰ and coronavirus¹¹. However, RNA recombination has not been found to date in viruses that infect plants. In fact, the lack of DI RNAs and the inability to demonstrate recombination in mixedly infected plants has been regarded as evidence that plants do not support recombination of viral RNAs. Here we provide the first molecular evidence for recombination of plant viral RNA. For brome mosaic virus (BMV), a plus-stranded, tripartite-genome virus of monocots, we show that a deletion in the 3' end region of a single BMV RNA genomic component can be repaired during the development of infection by recombination with the homologous region of either of the two remaining wild-type BMV RNA components. This result clearly shows that plant viruses have available powerful recombinatory mechanisms that previously were thought to exist only in animal hosts, thus they are able to adapt and diversify in a manner comparable to animal viruses. Moreover, our observation suggests an increased versatility of viruses for use as vectors in introducing new genes into plants.

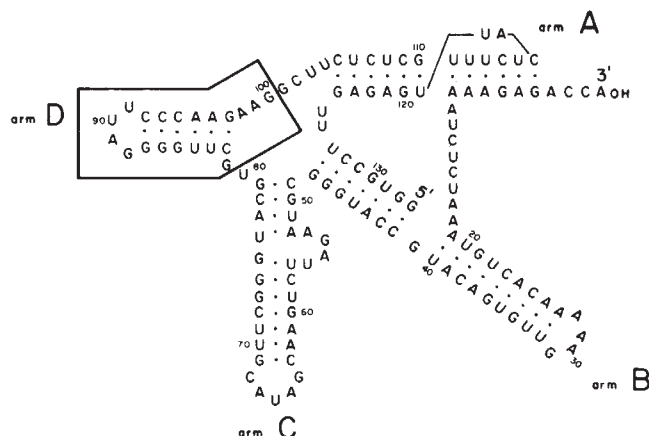


Fig. 1 Schematic representation of the postulated conformation of the tRNA-like structure at the 3' end of BMV RNA3 (after Rietveld *et al.*¹⁴). The open box indicates the location of the 20-nucleotide deletion (deletion m4) so that the entire D arm is absent from the resulting structure. This deletion, located between nucleotides 81 and 100, was originally introduced into a cDNA clone comprising the last 200 bases of BMV RNA3 as described in ref. 19 and subsequently transferred into the full-length cDNA clone of BMV RNA3 as described in ref. 21.

To study recombination between the individual BMV RNAs, we used wild-type RNA1 and RNA2 together with an engineered BMV RNA3 in which a sequence located in its 3'-proximal noncoding region had been deleted. The 3'-noncoding region for each of the three BMV RNAs extends for ~300 nucleotides, the last 200 of which are similar in sequence but not identical in the three RNAs¹². Their 3'-proximal 134 nucleotides are folded into a transfer RNA-like conformation^{13,14}, which is involved in initiation of BMV RNA replication^{15,16}, aminoacylation¹⁷ and adenylation¹⁸. The RNA3 mutant, designated m4, had been constructed previously by S₁ nuclease deletion of a 20-base long, stem-and-loop hairpin corresponding to arm D in its tRNA-like structure¹⁹ (bases 81–100; see Fig. 1).

RNAs 1 and 2, transcribed from cDNA clones of wild type (Madison M1 strain)²⁰, together with transcribed m4-type RNA3, are infectious to barley plants and progeny virus initially has the m4-type 3' region in both RNA3 and RNA4 (ref. 21). RNA4 is a subgenomic RNA derived during virus infection from the 5'-proximal one-third of negative-strand RNA3 (ref. 22). However, m4 RNA3 and RNA4 accumulated less well than their wild-type counterparts, probably because of modified activity of the m4-type 3' region, as determined *in vitro*^{19,21}. We therefore decided to test the possibility that, after a prolonged infection, the original m4 RNA3 might be outcompeted by a revertant form of RNA3 which had regained the deleted stem-and-loop region as a result of recombination with wild-type RNA1 or RNA2.

Time-course analysis of progeny viral RNA3 species from successively appearing leaves of plants inoculated with transcribed wild-type RNAs 1 and 2 and m4 RNA3 revealed a stepwise decrease of the m4 RNA3 over days 3–16 after inoculation (Fig. 2). The presence of the m4 deletion near the 3' end in RNAs 3 and 4 was determined by observation of a readily identifiable, 3'-terminal fragment released by partial digestion with ribonuclease T₁ (ref. 23). The disappearance with time of the 141-base-long m4-type fragment expected for m4 RNA3, and the parallel appearance of a 161-base fragment characteristic of wild-type RNA3 (compare lanes 2 and 4 of Fig. 2) indicated a gradual increase of an RNA3 variant that was more similar to wild-type RNA3.

To delineate the emergent RNA more precisely, we examined progeny RNA3 and RNA4 isolated from three individual barley plants on day 15 post-inoculation. In plant I we found that the variant RNA3 and RNA4 each migrated more slowly on agarose

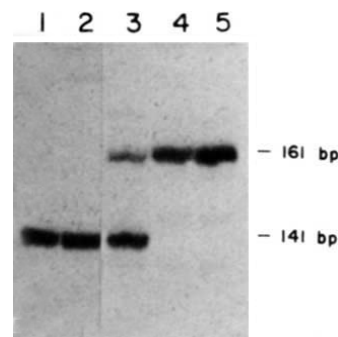


Fig. 2 Analysis by polyacrylamide gel electrophoresis for the presence of the m4 deletion in a 3'-terminal fragment released from progeny BMV RNA3 by limited digestion with RNase T₁. Lanes 5 and 1, standard T₁ fragments obtained from wild-type (161 base pairs (bp) long²³) or m4-type (141 bp) transcript RNA3, respectively. Lanes 2, 3 and 4, T₁ fragments from progeny BMV RNA3 isolated from the virus at 3, 10 and 16 days post-inoculation, respectively.

Methods. Barley seedlings were inoculated with a mixture of transcribed wild-type BMV RNAs 1 and 2 and the m4 RNA3 following the procedure described in ref. 20. Progeny viral RNA was isolated from virus extracted from 0.1 g of leaves of a single infected plant, as described elsewhere²⁷. The BMV RNA3 component was separated by electrophoresis on a low-melting point 1% agarose gel and a 3'-terminal fragment was released from the purified RNA3 by limited digestion with RNase T₁ as described elsewhere²³. To generate standard 161- and 141-bp T₁ fragments, both wild-type and m4 RNA3 were synthesized by transcription *in vitro* as described in ref. 28, and the fragments were released from transcripts with RNase T₁. After labelling of the 3' ends with ³²P-pCp and T₄ RNA ligase²⁹, the released RNA fragments were analysed on a 6% polyacrylamide/7 M urea sequencing gel and their positions were determined by autoradiography.

gel electrophoresis than m4-type and also wild-type RNAs 3 and 4 (see Fig. 3, lanes 1 and 2), indicating that they are larger than both m4-type and wild-type RNAs 3 and 4. There was no evidence of m4-type or wild-type RNA3 or RNA4. This pattern was unaltered after two passages of the virus through other barley plants. Sequence analysis of RNA3 from the emergent virus (designated mutant A; see Fig. 4 legend) indicated that the region corresponding to the last 267 bases of wild-type RNA3 had been replaced with the last 307 bases of wild-type RNA2. Thus, mutant A RNA3 had the deleted stem-and-loop region restored plus 39 more bases than wild-type RNA3 and 59 more bases than its m4 RNA3 progenitor.

In plant II, viral RNA3 and RNA4 co-migrated with wild-type BMV RNA3 (Fig. 3, lanes 4 and 5). Electrophoresis on a higher-resolution gel showed that the RNA3 was composed of at least two bands, the lower band containing most of the material (Fig. 3, lane 6, bands 3a and 3b). In plant III, gel electrophoresis revealed two bands each in the regions of RNA3 and RNA4 (compare lanes 7 and 10 of Fig. 3); a similar pattern was present after the first passage of the virus (lane 8), whereas after the second passage the upper band was absent (lane 9). Analysis on a higher resolution gel indicated that the lower band in the region of RNA3, present as a single band in lane 9, in fact consisted of at least two closely migrating bands (lane 11, bands 3c and 3d).

To identify the species present in RNA3 preparations obtained from plants II and III, a complementary DNA library was created and individual clones were sequenced in their 3'-noncoding regions, as described in Fig. 4 legend. Three sequences, corresponding to mutants designated B, C and D, were found in both libraries. In addition, a fourth sequence, corresponding to mutant E, was identified in the library of plant III. While four clones of mutant B, three clones of mutant C, eight of mutant D and one of mutant E were identified, cDNA clones corresponding to neither m4-type RNA3 nor the strictly wild-

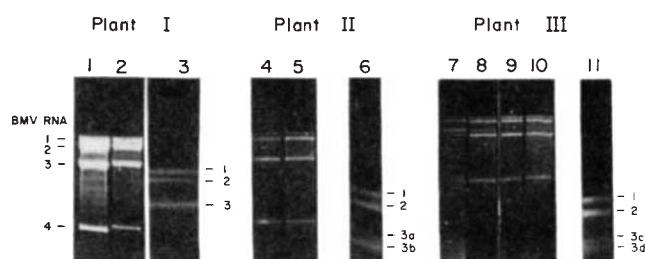


Fig. 3 Electrophoretic analysis of progeny BMV RNA extracted from three independently inoculated barley plants. Barley leaves were infected with transcribed wild-type RNAs 1 and 2 and m4 RNA3. Plants I and II: BMV RNA was extracted from leaves showing symptoms of infection, on day 15 after inoculation, and was analysed on 1.2% agarose gels (either 10 cm long, lanes 2 and 5; or 30 cm long, lanes 3 and 6). Plant III: viral RNA was obtained from leaves 15 days after inoculation with transcribed BMV RNAs (lane 7) as well as on day 15 after the first and second passages through barley (lanes 8 and 9, respectively; 10-cm gels). Lane 11, RNA from lane 9 analysed on a 30-cm gel. Lanes 1, 4 and 10, wild-type BMV RNA.

type 3' end sequence of BMV RNA3 were found. This last observation tends to exclude the possibility that a causal infection with wild-type BMV RNA3 contaminated the experiments.

The sequences of RNA3 in mutants B, C and D revealed recombination between RNA3 and RNA1 or RNA2 at a site in their homologous 3' end region. As these RNAs differ in only a small number of bases in this region (see Fig. 4 legend), the exact site of putative recombination could not be specified. We can conclude, however, that mutant B is a combination of RNA3 and RNA1 somewhere between bases 130 and 101, mutant C combines the RNA3 and RNA1 sequences somewhere between bases 175 and 131, and mutant D combines RNA3 and RNA2 somewhere between bases 205 and 101. Similar to mutant A, mutant E had its deleted stem-and-loop region restored and was larger than wild-type RNA3. Sequence analysis showed that the region corresponding to the last 206 bases of the wild-type RNA3 had been replaced by 215 3'-terminal nucleotides from wild-type RNA2 (see Fig. 4).

The structure of these five mutants suggests strongly that one or more recombination events have occurred between the 3'-noncoding regions (within or outside the homologous sequences) of individual BMV RNAs, resulting in the restoration of the deleted hairpin in progeny RNA3. Clearly, that stem-and-loop region is not essential for infectivity but its presence nevertheless confers a selective advantage. In mutants A and E recombination outside the closely homologous region resulted in emergent noncoding regions larger than wild-type RNA3, indicating that a degree of structural variability is permissible there. In mutants B, C and D the recombination event(s) occurred within the homologous region and resulted in sequences of wild-type lengths only.

The results described here demonstrate that recombination between viral RNAs occurs in plant cells at a relatively high frequency and at various positions on the RNA. As with animal RNA viruses, the mechanism of recombination between the BMV sequences is unknown. Two diverse mechanisms have been suggested for RNA genomes: (1) Enzymatic cutting and re-ligation. (2) A 'copy-choice', during replication, between adventitiously proximal templates. The latter mechanism has been favoured for recombination in picornaviruses²⁴, negative-strand viruses^{5,10} and alpha viruses⁷.

The recent discoveries of remarkable sequence similarities among encoded proteins of diverse plant RNA viruses^{25,26} suggest that recombination may be a common phenomenon during synthesis of plant viruses. Like other processes of natural mutagenesis, recombination between plant viruses may render the progeny more adaptable to new environments or hosts.

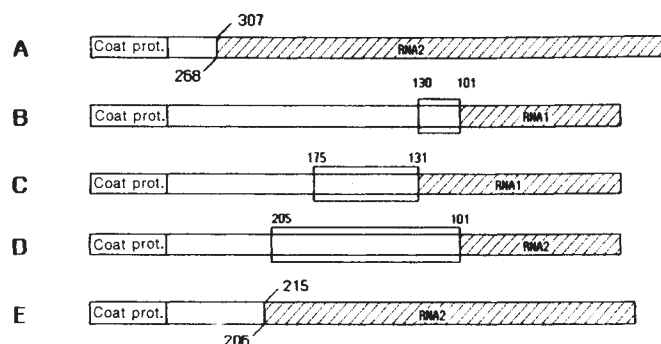


Fig. 4 Schematic representation of BMV RNA3 recombinants identified by sequence analysis of BMV RNA3 preparations obtained from plants I-III. Mutant A was obtained from plant I; mutants B-E were identified in plants II and III. The figure shows the 3' region of RNA3 for the five mutants downstream to the coat protein open reading frames (ORFs). The open regions adjacent to the ORFs represent wild-type BMV RNA3 sequences. The cross-hatching indicates regions identical to wild-type RNA1 or RNA2, possessing the stem-and-loop region of arm D, and also differing from wild-type RNA3 at the following base positions: RNA1 has distinctive bases at positions 101, 44 and 43 while RNA2 has distinctive bases at positions 101 and 44 and has a point deletion corresponding to base 74 of RNA3 (refs 13, 20). The boxes enclose regions in which the recombination event occurred, assuming a recombination mechanism involving a single event. Sequences of wild-type RNAs 1, 2 and 3 are identical in these regions.

Methods. Mutant A RNA3 was sequenced directly by a chain-termination (dideoxy) technique with RNA-dependent DNA polymerase (reverse transcriptase³⁰). Briefly, 2 µg of RNA3, obtained after fractionation on a sucrose gradient³¹, was hybridized with 1 µg of either the 15-mer d(GAGATTTCTCTGGT), complementary to nucleotide positions 1-15, or the 15-mer d(TCTCACAGATCCTCG), complementary to nucleotides 129-143, and these primers were extended with 10 units of reverse transcriptase (Life Science) in the presence of dideoxy nucleoside triphosphates. RNA3 preparations obtained from plants II and III (mutants B-E) were sequenced as cDNA clones in an M13mp19 vector by a chain-termination (dideoxy) technique with a synthetic 17-mer primer (BioLabs) and DNA polymerase large fragment (Klenow)^{32,33}. Two cDNA libraries were created for BMV RNA3 preparations obtained either from primary infection in plant II or after a second passage of the virus in plant III. The first cDNA strand was synthesized with reverse transcriptase as described elsewhere³⁴, using the d(TAGAGATTTCTCTGGT) primer complementary to the last 17 nucleotides of BMV RNA3. After purification on a 1 × 20 cm Sepharose CL2B column, the second strand was obtained by means of a replacement reaction with RNase H, *Escherichia coli* DNA polymerase and *E. coli* DNA ligase³⁵. After treatment with T₄ DNA polymerase (blunt-ending), the resulting double-stranded cDNA preparation was cloned into a *Sma*I restriction site of M13mp19 vector and the sequence of the corresponding single-stranded M13 DNA templates was determined by a chain-termination procedure using the Klenow fragment of *E. coli* DNA polymerase and a synthetic 17-mer (BioLabs) as primer.

Conversely, RNA recombination between plant viral RNAs may be important for the preservation and stabilization of crucial functional sequences of multipartite genome viruses. Because it facilitates the association of unrelated genes, recombination also represents an attractive candidate for the mechanism of origin of viruses and for the insertion of new genes into existing viruses.

We thank Ola Dzianott-Bujarska for technical assistance, and G. Duke, P. Shaklee and P. Ahlquist for valuable advice with respect to cDNA cloning. This work was supported by NIH research grants AI01466 and AI15342 and Career Award AI21942.

Received 21 January; accepted 18 March 1986.

- Cooper, P. D. *Virology* **35**, 584-596 (1968).
- King, A. M. Q., McCahon, D., Slade, W. R. & Newman, J. W. I. *J. Virol.* **41**, 66-77 (1982).

3. Tolskaya, E. A., Romanova, L. A., Kolesnikova, M. S. & Agol, V. I. *Virology* **124**, 121-132 (1983).
4. Emini, E. A., Leibowitz, J., Diamond, D. C., Bonin, J. & Wimmer, E. *Virology* **137**, 74-85 (1984).
5. Lazzarini, R. A., Keene, J. D. & Schubert, M. *Cell* **26**, 145-154 (1981).
6. Jennings, P. A., Finch, J. T., Winter, G. & Robertson, J. S. *Cell* **34**, 619-627 (1983).
7. Monroe, S. S. & Schlesinger, S. J. *Virology* **49**, 865-872 (1984).
8. Stark, C. & Kennedy, S. I. T. *Virology* **89**, 285-299 (1978).
9. Lai, M. M. C. *et al.* *J. Virol.* **56**, 449-456 (1985).
10. Plotch, S. J., Bouloy, M., Ulmanen, I. & Krug, R. M. *Cell* **23**, 847-858 (1981).
11. Baric, R. S., Stohlman, S. A., Razavi, M. K. & Lai, M. M. C. *Virus Res.* **3**, 19-33 (1985).
12. Ahlquist, P., Dasgupta, R. & Kaesberg, P. *J. molec. Biol.* **172**, 369-383 (1984).
13. Ahlquist, P., Dasgupta, R. & Kaesberg, P. *Cell* **23**, 183-189 (1981).
14. Rietveld, K., Pleij, W. A. & Bosch, L. *EMBO J.* **2**, 1079-1085 (1983).
15. Ahlquist, P., Bujarski, J. J., Kaesberg, P. & Hall, T. C. *Pl. molec. Biol.* **3**, 37-44 (1984).
16. Miller, W. A., Bujarski, J. J., Dreher, T. W. & Hall, T. C. *J. molec. Biol.* **187**, 537-546 (1986).
17. Hall, T. C. *Int. Rev. Cytol.* **60**, 1-26 (1979).
18. Joshi, R. L., Joshi, S., Chapeville, F. & Haenni, A. L. *EMBO J.* **2**, 1123-1127 (1983).
19. Bujarski, J. J., Dreher, T. W. & Hall, T. C. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5636-5640 (1985).
20. Ahlquist, P., French, R., Janda, M. & Loesch-Fries, L. S. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7066-7070 (1984).
21. Bujarski, J. J., Ahlquist, P., Hall, T. C., Dreher, T. & Kaesberg, P. *EMBO J.* (submitted).
22. Miller, W. A., Dreher, T. W. & Hall, T. C. *Nature* **313**, 68-70 (1985).
23. Bastin, M., Dasgupta, R., Hall, T. C. & Kaesberg, P. *J. molec. Biol.* **103**, 737-745 (1976).
24. Cooper, P. D., Steiner-Pryor, A., Scotti, P. D. & DeLong, D. J. *Gen. Virol.* **23**, 41-49 (1974).
25. Haseloff, J. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **81**, 4358-4362 (1984).
26. Cornelissen, B. & Bol, J. *Pl. molec. Biol.* **3**, 379-384 (1984).
27. Shih, D. S., Lane, L. C. & Kaesberg, P. *J. molec. Biol.* **64**, 353-362 (1972).
28. Ahlquist, P. & Janda, M. *Molec. cell. Biol.* **4**, 2876-2882 (1984).
29. England, T. & Uhlenbeck, O. *Nature* **275**, 560-561 (1978).
30. Zimmer, D. & Kaesberg, P. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4257-4261 (1978).
31. Kiberstis, P. A., Loesch-Fries, L. S. & Hall, T. C. *Virology* **112**, 804-808 (1981).
32. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463-5467 (1977).
33. Biggin, M. D., Gibson, T. J. & Hong, G. F. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3963-3965 (1983).
34. Fields, S. & Winter, G. *Cell* **28**, 303-313 (1982).
35. Okayama, H. & Berg, P. *Molec. cell. Biol.* **2**, 161-170 (1982).

A tobacco mosaic virus-induced tobacco protein is homologous to the sweet-tasting protein thaumatin

Ben J. C. Cornelissen,

Rob A. M. Hooft van Huijsduijnen & John F. Bol

Department of Biochemistry, State University of Leiden,
PO Box 9505, Leiden, The Netherlands

Infection of tobacco plants with tobacco mosaic virus (TMV) results in an increase in the activities of several enzymes and induces the *de novo* synthesis of about 10 proteins that are protease-resistant and soluble at pH 3. These proteins accumulate in the intercellular leaf space^{1,2}. The appearance of pathogenesis-related (PR) proteins is closely associated with the phenomenon of 'systemic acquired resistance' and it has been suggested that such proteins have an antiviral function^{3,4}. Previously, we cloned complementary DNAs to the messenger RNAs for the three smallest PR proteins, PR-1a, -1b and -1c, and these clones were used to show that there is an increase of more than 100-fold in the concentration of PR-1 mRNAs following TMV infection of tobacco^{5,6}. Here, we describe the cDNA cloning of another mRNA whose synthesis is induced by TMV infection. Sequencing of the cDNA showed that the encoded protein is highly homologous to thaumatin, the intensely sweet-tasting protein from the fruits of the moncot *Thaumatococcus daniellii* Benth, a West African rainforest shrub. The conservation of a gene encoding a thaumatin-like protein in tobacco suggests that the encoded protein may have a more general function than that of being sweet-tasting.

Poly(A)-containing RNA from TMV-infected Samsun NN tobacco was used as a template to construct a cDNA library; 1,400 transformants were screened by a differential hybridization procedure, using ³²P-labelled DNA complementary to poly(A) RNA from healthy or TMV-infected tobacco as probes. Forty transformants were found to correspond to TMV-induced mRNAs and an analysis of their inserts showed that they could be divided into six clusters, with cross-hybridization occurring within but not between the clusters. Details of the cloning and selection procedure will be given elsewhere (R.A.M.H. *et al.*, in preparation).

Fig. 1 Northern blot loaded with poly(A) RNA from healthy tobacco (H), tobacco sprayed with salicylic acid (S) and tobacco infected with TMV (T). The blot was probed with ³²P-labelled PROB 12. Samsun NN tobacco plants were sprayed with salicylic acid (5 mM, adjusted to pH 7) on three successive days before RNA extraction. RNA was extracted from TMV-infected tobacco plants 7 days after inoculation. The method of extraction, Northern blotting and the cloning of PROB 12 are described in ref. 6.



One cluster was represented by a single clone, PROB 12, which was used to probe the Northern blot shown in Fig. 1. The gel used to make the blot was loaded with poly(A) RNA from healthy tobacco (lane H), tobacco sprayed with salicylic acid (lane S), and tobacco infected with TMV (lane T). Salicylic acid is known to induce the synthesis of several PR proteins, notably proteins 1, 2 and N². The mRNA corresponding to PROB 12 occurs at a low level in healthy tobacco, is not induced by treatment with salicylic acid, but is strongly induced by TMV infection. This mRNA is estimated to be 1,000-1,100 nucleotides long. Sequencing studies showed that the insert in PROB 12 is 845 base pairs (bp) long. As no poly(A) tract was found in the cDNA, the 3' end of the mRNA is probably not represented in the clone. Figure 2 shows that the insert contains an open reading frame for a protein of 226 amino acids, flanked by 5'- and 3'-noncoding regions of 3 and 164 nucleotides, respectively.

The nucleotide sequence of the insert of PROB 12 was compared, by computer analysis, with plant sequences stored in a databank (Genbank). Extensive homology was found with the mRNA for thaumatin, the sweet-tasting protein that occurs in the arils of *Thaumatococcus daniellii* Benth, a West African shrub⁷. At least five different forms of thaumatin (I, II, III, b and c) can be isolated, all of which are almost 100,000 times sweeter than sucrose on a molar basis⁸. The most abundant forms are thaumatin I and II, polypeptides of 207 amino acids each that differ at only five positions⁹. The mature protein is derived from preprothaumatin by removal of an amino-terminal signal peptide of 22 amino acids and an acidic carboxy-terminal extension of 6 amino acids⁷. In Fig. 3, preprothaumatin II is aligned with protein encoded by PROB 12; the amino-acid sequence homology between the two proteins is 65%. In addition, there are several conserved amino-acid changes; when these are taken into account, the homology is more than 70%. The Ala-Ala sequence, which represents the site of cleavage of the signal peptide, is conserved in both proteins. However, except for their hydrophobic nature, there is little similarity between the signal peptides of the two proteins, and the tobacco protein lacks the C-terminal extension of the thaumatin precursor. Because of its homology to thaumatin, we provisionally refer to the PROB 12-encoded protein as a thaumatin-like (or TL) protein of tobacco.

The alignment shown in Fig. 3 suggests that PROB 12 contains the complete coding region for the precursor of the TL protein. A possible polyadenylation signal in TL mRNA (underlined in Fig. 1) is located 80 bp upstream from the 3' end of the cDNA insert. There may be additional polyadenylation signals in the TL mRNA sequence that are not represented in PROB 12. Three potential polyadenylation signals have been found in thaumatin mRNA⁷.

The relative molecular mass (M_r) of the mature TL protein of tobacco is 21,596. It is unknown whether the TL protein

```

M N F L K S F P F Y A F L C F G Q Y F
AAUUGAACUUCUCAAAGGCUUCCUUAUUGCCUUGUUGGCAUUCU
10      20      30      40      50      60
V A V T H A A T F D I V N Q C T Y T V W
GUAGCUGUUAACUAGCUGGCAUUCUUAUGCAACCAUAGCAGCAGUCUGG
70      80      90      100     110     120
A A A S P G G G X Q L N S G Q S W S I N
GCCGCGGCUUCCUAGGAGGAGGAGCUCACGCGGCAUUGGAGCAUUAAC
130     140     150     160     170     180
V N P G T V Q A R I W G R T N C N F D G
GUGAACCCAGGAACAGCAGGUCGCAUUGGCGCAACCAUUGCAUUCGAUGG
190     200     210     220     230     240
S G R G N C E T G D C N G M L E C Q G Y
AGUGGCGGAGGUAUUGAGAGCUGGAGCAGUAAACGCGGCAUUGGAGCAU
250     260     270     280     290     300
G K P P N T L A E F A L N Q P N Q D F V
GGUAAACACCAACACUUAUAGCUGAAUUGCAUUAACGCCCAUAGCAGCAGC
310     320     330     340     350     360
D I S L V D S F N I P M E F S P T N G G
GACAUUCUCUUGUUGAGGUAUUAACUACCCAUUGGAUUCAGCCCAUUAUGGCGG
370     380     390     400     410     420
C R N L R C T A P I N E Q C P A Q L K T
UGCGUAUUCUUAUGAGCAGCAGCAUUAUAGGCAUUGGCGGCAUUGAAACA
430     440     450     460     470     480
Q G G C N N P C T V I K T N E F C C T N
LAAGGUGGAGUUAACCAUUGCUGUUAUUAACCAUUAUUAUUGUUAUUAU
490     500     510     520     530     540
G P G S C G P T D L S R F F K A R L P D
GGGCGUGGAGUUAUGGCGGCUUAGGAGGAGUUAUUAUUAUUAUUAUUAU
550     560     570     580     590     600
A Y S Y P Q D D P P S L F T C P P G T N
GCUUAUAGUUAUCCAGGAGUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAU
610     620     630     640     650     660
Y R V Y F C P *
UACAGGUGUUGUUCUUGGCGUUAUUAUUAUUAUUAUUAUUAUUAUUAU
670     680     690     700     710     720
AGUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAUUAU
730     740     750     760     770     780
CAAGAGGAGGCGGUGGUGCAGAUUUCUUGGUGUGUUGUUGUUGUUGUUG
790     800     810     820     830     840
GUUUU

```

Fig. 2 Nucleotide sequence of the coding strand of PROB 12 cDNA. The amino-acid sequence corresponding to the open reading frame is indicated (one-letter code). The polyadenylation signal in the 3'-noncoding region is underlined.

corresponds to a known PR protein, for example, P or Q (each of estimated $M_r \sim 27,000$; ref. 5). In preliminary immunodiffusion experiments, purified PR proteins, or a total protein extract from TMV-infected tobacco, did not react with an antiserum to thaumatin. The PR-1 proteins are derived from their precursors by the removal of a 30-amino-acid signal peptide, probably during excretion into the intercellular space of the tobacco leaf^{5,6}. The evidence for a signal peptide in the TL primary translation product also suggests that the TL protein is translocated through a membrane. The thaumatins are known to accumulate in intracellular vesicle-like organelles¹⁰. If, by analogy, the TL protein is not localized extracellularly in tobacco, it may not belong to the PR class of proteins.

The extreme sweetness of thaumatin, which is only recognized by man and Old World monkeys, may contribute to the spreading of the seeds of *T. daniellii* Benth. The conservation of a thaumatin-like gene in tobacco and possible other dicotyledonous plants suggests that in these plants the encoded protein performs a more general function. Thaumatin ($pI = 12$) has a high proportion of basic residues and modification studies indicate that some lysine residues are important for the sweet taste^{11,12}. In the tobacco TL protein there is an excess of acidic residues over basic residues, which may alter its properties with respect to taste perception. Recently, the three-dimensional structure of thaumatin I has been deduced at 3.1 Å resolution¹³. It will be interesting to compare the predicted TL protein structure with the thaumatin backbone.

The presence of two very small introns at different positions in several thaumatin genes has led to the suggestion that the genes encoding thaumatin are structurally similar to those encoding storage proteins such as zein (maize) and glycinin (soybean)⁹. The availability of PROB 12 offers the possibility of monitoring for a tissue-specific expression of the TL gene during the life cycle of tobacco. A model has been proposed in which the burst of ethylene production that is evoked by TMV infection of tobacco, is responsible for the induction of PR proteins and of several enzymes¹. In this respect it is noteworthy that ethylene production increases considerably during specific developmental processes such as fruit ripening.

```

PROB 12      M N F L K S F P F Y A F L C F G Q Y F Y A T H A
THAU 11      M A A T T C F F L F F F L L L L T L S R A

PROB 12      A T F D I V N Q C T Y T V W A A S P G - - - - G G R Q
THAU 11      A T F D I V N R C Y T V W A A S K G D A A L D A G G R Q

PROB 12      L N S G G S W S I N V N P G T Y Q A R I W G R T N C N F D G
THAU 11      L N S G S W S I N V E P G T K G G K I W A R T D C Y F D G

PROB 12      S G R G N C E T G D C N G M L E C Q G Y G K R F G R P P T L A E F
THAU 11      S G R G T C R T G D C G G L L Q G K R F G R P P T L A E F

PROB 12      A L N Q P N Q D F Y D I S L Y D G F N I P M E F S P T N G G
THAU 11      S L N Q Y G K D Y T D I S N I K G F N Y P M D F S P T T R G

PROB 12      C R N L R C T A P I N E Q C P A Q L K T - G G C N N P C T
THAU 11      C R G Y R C A A D I Y G C P A K L K A P G G C N D A C T

PROB 12      V I K T N E F C C T T N G P G S C G P T D L S R F F K A R C P
THAU 11      V F Q T S E Y C C T T G K - - C G P T E Y S R F F K R L C P

PROB 12      D A Y S Y P Q D D P P S L F T C P P G T N Y R V Y F C P
THAU 11      D A F S Y Y L D K R T T V - T C P G S S Y R V Y F C P

```

Fig. 3 The amino-acid sequence encoded by the open reading frame in PROB 12, aligned with the preprothaumatin II sequence⁷. Amino acids that are identical in the two sequences are boxed. The arrows indicate the cleavage sites that are used to remove the N-terminal signal peptide and the C-terminal extension from preprothaumatin II.

We thank Dr A. M. Ledebøer (Unilever Research Laboratory, Vlaardingen, The Netherlands) for providing an antiserum against thaumatin. This work was sponsored in part by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Received 26 February; accepted 21 March 1986.

1. Van Loon, L. C. in *Active Defense Mechanisms in Plants* (ed. Wood, R. K. S.) 247-274 (Plenum, New York, 1982).
2. Van Loon, L. C. *Pl. molec. Biol.* **4**, 111-116 (1985).
3. Kassanis, B., Gianinazzi, S. & White, R. F. *J. gen. Virol.* **23**, 11-16 (1974).
4. Van Loon, L. C. *Virology* **67**, 566-575 (1975).
5. Hooft van Huijsduijnen, R. A. M. *et al. EMBO J.* **4**, 2167-2171 (1985).
6. Cornelissen, B. J. C., Hooft van Huijsduijnen, R. A. M., Van Loon, L. C. & Bol, J. F. *EMBO J.* **5**, 37-40 (1986).
7. Edens, L. *et al. Gene* **18**, 1-12 (1982).
8. Higginbotham, J. D. in *Developments in Sweeteners-I* (eds Hough, C. A. M., Parker, K. J. & Vliots, A. J.) 87-123 (Applied Science Publications, London, 1979).
9. Ledebøer, A. M., Verrips, C. T. & Dekker, B. M. M. *Gene* **30**, 23-32 (1984).
10. Edens, L. & Van der Well, H. *Trends Biotechnol.* **3**, 1-4 (1984).
11. Van der Wel, H. & Bel, W. *Chem. Senses* **2**, 211-218 (1976).
12. Morris, R., Cagan, R., Martenson, R. & Deibler, G. *Proc. Soc. exp. Biol. Med.* **157**, 194-318 (1978).
13. De Vos, A. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 1406-1409 (1985).

Nature Product Review

MANUFACTURERS and distributors of scientific equipment are reminded that the following special features are appearing in July and August:

- 3 July:** **Microscopy/Image analysis**, covering all aspects of microscope hardware and techniques as well as latest developments in image analysers.
- 3 July:** **Immunology**. Also in this issue, details of exhibits at the Toronto International Congress of Immunology, to be held on 7-10 July.
- 4 August:** **New on the Market:** A selection of recently introduced laboratory equipment with the accent on novelty.
- 14 August:** **Computers in the laboratory:** Information technology and computer services in the modern laboratory.

Press releases, photographs and comments on these features should be addressed to the Editor in *Nature's* London office or to:

Karen Wright,
Nature,
1134 National Press Building,
Washington, DC 20045, USA

Was there 26-Myr periodicity of extinctions?

HOFFMAN¹ has questioned our claim² that extinction events during the past 250 Myr exhibit a 26-Myr periodicity. Hoffman argues that the apparent periodicity originates not from any evolutionary signal but from arbitrariness in the choice of taxonomic level, treatment of data and selection of geologic timescales in our analysis. His letter succeeds in outlining many problems inherent in working with data from the fossil record. However, we find his conclusion unconvincing because there is no hypothesis-testing. In fact, when statistical tests and independent criteria are applied, we find that Hoffman's results support our contention of periodicity better than our initial analyses.

In our initial paper² we analysed a set of data on fossil marine families that was highly culled in order to enhance the signal-to-noise ratio. Hoffman implies that this culling may have induced an appearance of periodicity. However, our subsequent analyses^{3,4} indicate that this culling had no important consequences and an evident 26-Myr periodicity remains when a variety of extinction metrics (including rate and probability of extinction) are computed from the complete, uncultured data set. Indeed, Hoffman's own measures of extinction intensity, summarized in his Table 1, also show this if consistent patterns rather than random noise are sought. Ten of Hoffman's 43 stages exhibit peaks (local maxima) in more than half of the 21 metrics (including the unillustrated regression residuals for number of family extinctions per stage). The ten stages are the Guadalupian, Olenekian, Norian, Pliensbachian, Bajocian, Tithonian, Cenomanian, Maestrichtian, late Eocene and middle Miocene. Of these, all but the Olenekian, Bajocian and middle Miocene have been independently recognized as containing extinction events by palaeontologists using detailed biostratigraphic data on species and genera^{3,5}, and only one other stage (the Pliocene) in the Guadalupian-to-Recent interval has been so recognized. The probability that the family data would encompass 7 of 8 events in 10 random peaks among 43 stages is <0.00003 , strongly indicating that the consistent peaks among Hoffman's metrics result from evolutionary variation rather than artefact. This conclusion is further supported by the good correlation between familial and generic^{4,6} extinctions throughout the 43 stages. (For per cent extinction, $r = 0.893$ and for probability of extinction $r = 0.877$.)

Statistical tests similar to those used in our initial paper indicate that the 10 stages listed above exhibit a significant ($p < 0.002$) nonrandomness at a 26-Myr period.

Tests of this periodicity result in a standard deviation of differences between observed and expected peaks of 3.28 Myr using the Harland *et al.* timescale⁷ and 5.53 Myr using the Odin timescale⁸; both fits are better than those obtained in our original analysis². Fits with even higher significance are obtained if only the four most recent, and therefore most reliably dated⁹, stages are tested⁴.

Why did Hoffman conclude that the familial data appeared random with respect to extinction? It may have been because he used a necessary but not sufficient criterion for randomness. He argued that if random, the extinction metrics should display an average frequency of one peak in four stages; because our data displayed this frequency, he concluded that they were random. But there is a great difference between an average frequency of occurrence and a regular occurrence. A fair coin will, on average, come up heads once in two flips, but if it comes up heads regularly every other flip, we should suspect its fairness. In our analysis of the extinction data, our randomization test² actually converted the data into random walks with the same frequency of maxima as in the observed time series. Yet, when compared to these random walks, we found that the observed distribution of maxima was significantly ($p < 0.01$) more uniform than in the random walks. Thus, we rejected the hypothesis of randomness in favour of periodicity. We suggest that Hoffman might have done the same if he had performed the proper statistical tests.

J. JOHN SEPKOSKI JR
DAVID M. RAUP

*Department of the Geophysical Sciences,
University of Chicago,
5734 S. Ellis Avenue,
Chicago, Illinois 60637, USA*

- Hoffman, A. *Nature* **315**, 659–662 (1985).
- Raup, D. M. & Sepkoski, J. J. *Jr Proc. natn. Acad. Sci. U.S.A.* **81**, 801–805 (1984).
- Sepkoski, J. J. Jr & Raup, D. M. in *Dynamics of Extinction* (ed. Elliott, D.) 3–36 (Wiley, New York, 1986).
- Raup, D. M. & Sepkoski, J. J. *Jr Science* **231**, 833–836 (1986).
- Sepkoski, J. J. *Jr Spec. Pap. geol. Soc. Am.* **190**, 283–289 (1982).
- Sepkoski, J. J. Jr in *Patterns and Process in the History of Life* (eds Raup, D. M. & Jablonski, D.) (Springer, Berlin, in the press).
- Harland, W. B. *et al. A Geologic Time Scale* (Cambridge University Press, 1983).
- Odin, G. S. (ed.) *Numerical Dating in Stratigraphy* (Wiley, New York, 1982).
- Maddox, J. *Nature* **315**, 627 (1985).

HOFFMAN¹ and Hoffman and Ghiold² recently presented a statistical argument that has been widely regarded^{3–5} as casting serious doubt upon Raup and Sepkoski's⁶ proposed 26-Myr extinction periodicity. Here I show that their conclusion is false and reflects an incorrect application of their own neutral model. When properly applied, the model actually supports Raup

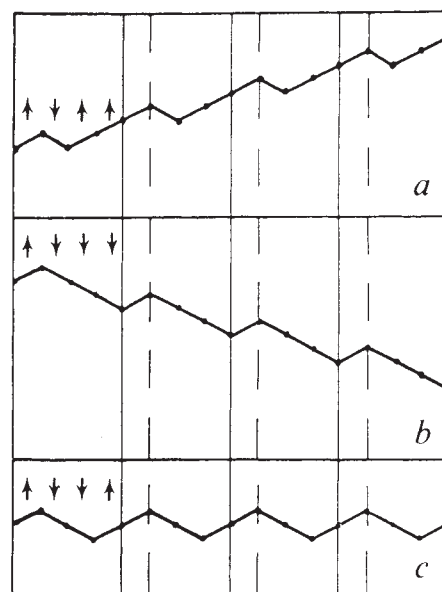


Fig. 1 The leftmost column shows the three ways of producing exactly one local extinction maximum in the second of a sequence of 4 stages. These are the unit sequences referred to in the text. Arrows indicate the direction of change (increase or decrease) in the extinction metric in each unit sequence. The unit sequences are repeated, producing three long periodic sequences of period 4. Although *a*, *b* and *c* each involve repetition of a single unit sequence, any unit sequence may follow any other and still create a periodic series. The magnitudes of the changes in extinction metric are ignored.

and Sepkoski's position and underscores the need for a causal explanation.

Hoffman and Hoffman and Ghiold's argument is as follows:

- (1) Given Raup and Sepkoski's definition of mass extinction as any local maximum of extinction metric; that is, any increase followed by decrease, and
- (2) Given that the probabilities of increase and decrease in extinction metric both equal 0.5, and
- (3) Given that the average stage length in the late Phanerozoic is 6.4 Myr, then the following conclusions hold:
 - (i) The probability that any particular stage is a local extinction maximum is equal to $(0.5)^2 = 0.25$.
 - (ii) Local extinction maxima are to be expected, on average, every four stages or every $4 \times 6.4 = 25.6$ Myr.
 - (iii) "The appearance of approximately 26 million year periodicity is inevitable."²

Conclusions i and ii are true. However, conclusion iii does not follow from premises 1–3 above and is false. The mistake is clarified by the following: Conclusion ii in more precise language, means that in a large number of samples of four stages, the arithmetic mean number of mass extinctions per sample will be 1. However, for an extinction pattern to be periodic with a period of four stages requires that every fourth and only every fourth stage

be a local extinction maximum. As these two statements are not equivalent conclusion ii does not guarantee an extinction periodicity of 4 stage-lengths, and the neutral model does not predict that a 26-Myr periodicity is "inevitable". Conclusion iii is false.

To calculate the probability of producing an extinction periodicity of 4 stage lengths by a random process under the neutral model, one must determine the number of ways of producing an extinction periodicity of this period that persists for n stages, and multiply this by the probability of generating any sequence n stages in duration. Let us treat a periodic sequence of period 4 and duration n as a series of unit sequences four stages in duration. In order for such a series to be periodic, each unit sequence in the series must have exactly one local extinction maximum, and these maxima must be in the same position. There are only three unit sequences that have the local maximum at the second stage (Fig. 1). Thus there are only three infinitely long periodic sequences of period 4 that can be built by infinite repetition of the same unit sequence. But since the conditions for periodicity allow any of the three unit sequences of Fig. 1 to precede or follow any other, it follows that there are exactly $3^{n/4}$ possible ways of producing a periodicity of period 4 with the extinction peak at the second stage. Because there are actually 4 different positions where the extinction peak may occur, corresponding to shifts of phase, there are a total of $4 \times 3^{n/4}$ possible periodic sequences of length n and period 4. The probability of generating any particular sequence of length n under the neutral model is 0.5^n ; therefore the probability of generating an extinction periodicity of duration n and period 4 is $4 \times 3^{n/4} \times 0.5^n$.

Applying this formula to the 39-stage periodicity reported by Raup and Sepkoski⁶, the probability of an extinction event occurring every 4 stages (~ 26 Myr) under the neutral model is $4 \times 3^{39/4} \times 0.5^{39} = 3.3 \times 10^{-7}$. For a 43-stage periodicity, as proposed more recently⁷, the probability is 6.1×10^{-8} . Thus the probability, under the neutral model, of the periodicity being a simple artefact of Raup and Sepkoski's definition of mass extinction and of the average duration of stratigraphic stages is essentially zero.

The neutral model, like all models, is an oversimplification of nature. Thus, even my exact solution is only an approximate assessment of the probability of producing the observed pattern by chance. In fact, the complexities of the real palaeontological record would render an exact analytical calculation of probability extremely complex, if not impossible. Accordingly, Raup and Sepkoski employ a non-parametric randomization technique to allow them to include real-world complexities

implicitly in the approximation of a probability value. The neutral model and randomization yield consistent results, and both support the conclusion that the proposed periodicity is real and needs to be explained.

I thank I. J. Good for discussion of the probability calculation.

NORMAN L. GILINSKY

Department of Geological Sciences,
Virginia Polytechnic Institute and
State University,
Blacksburg, Virginia 24061, USA

1. Hoffman, A. *Nature* **315**, 569–562 (1985).
2. Hoffman, A. & Ghiold, J. *Geol. Mag.* **122**, 1–4 (1985).
3. Maddox, J. *Nature* **315**, 627 (1985).
4. Lewin, R. *Science* **229**, 640 (1985).
5. *Scient. Am.* **253**(3), 52–53 (1985).
6. Raup, D. M. & Sepkoski, J. J. Jr. *Proc. natn. Acad. Sci. U.S.A.* **81**, 801–805 (1984).
7. Sepkoski, J. J. Jr & Raup, D. M. in *Dynamics of Extinction* (ed. Elliot, D.) Wiley, New York, in the press.

STATISTICAL testing, essential to the effective argument of controversies based on data and hypotheses, depends on distributions of random processes. Raup and Sepkoski¹ present data describing the ratio of marine families believed to have become extinct during each of 39 stratigraphic stages in the past 250 Myr. Ratios record the number of extinct to extant families, per stage, exclusive of families extant today or during the past 11.3 Myr. On the basis of these data, Raup and Sepkoski¹ have suggested that these extinction ratios have varied with regular periodicity. Hoffman² proposed a non-periodic random model, which formed the basis of his criticism of the work of Raup and Sepkoski. Hoffman's argument rests on the claim that the empirical mean time between peaks of extinction ratios reported by Raup and Sepkoski is not very different from the mean expected under this random model. However, the variance, and not the mean, tests the hypothesis of periodicity.

To compare the empirical variance with that expected under Hoffman's model, we have simulated the distribution of the statistic used to estimate variance, which should be the basis of Hoffman's criticism. The problem of the expected variance in the spacing of peaks, given this random process, can be restated as follows: what is the variance in waiting time to a reversal from increase to decrease in extinction ratio? For each time interval the extinction ratio either increases or decreases relative to the preceding interval. An interval of increase is recognized as a peak (that is, a local maximum in extinction rate) if the time interval immediately following is an interval of decrease. The number of time intervals between successive peaks (measured by counting the peak at the beginning and all time intervals until but not including the next peak) is the length of time, L , between successive peaks. In the data of Raup and Sepkoski¹ the lengths

of these inter-peak times are: 2, 4, 4, 2, 2, 3, 3, 4, 5, 5, 4. The average of these lengths is 3.454, which would be the approximate period of the hypothesized periodicity.

To retain equivalency with the non-periodic random process described by Hoffman, we assumed, as he did, that (1) the probabilities of increase and decrease are equivalent and equal to 0.5, (2) the probabilities are independent, and (3) the time steps are of equal duration. To test whether extinction rate is a periodic function of time, we need to consider the distribution of the random variable L , its mean and variance, and the distribution of estimators of its mean and variance calculated from the sampling procedure used to observe the data of Raup and Sepkoski¹. The distribution of L is

$$\Pr(L=r) = \sum_{i=1}^{r-1} (1-p)^i p^{(r-1)},$$

$$r = 2, 3, 4, \dots$$

When $p = 0.5$, as suggested by Hoffman, the probability of an inter-peak time of r intervals is

$$\Pr(L=r) = \frac{(r-1)}{2^r}$$

The expected mean and variance of L are both equal to 4. Most of this distribution occurs in the inter-peak lengths 2–5, with frequencies of 0.25, 0.25, 0.19 and 0.125, respectively. If the observed inter-peak lengths are more uniform than would be expected under Hoffman's random hypothesis, this would support the idea that extinction rate is a periodic function of time. Thus, if the variance estimated from the data were significantly less than the value of this estimator of the variance of L expected under Hoffman's hypothesis, then periodicity would be supported; otherwise, periodicity would seem less plausible. The observed variance among the inter-peak lengths of the Raup and Sepkoski¹ data is 1.19. To determine its significance, however, we need to know the distribution of the variance estimates according to Hoffman's null hypothesis, based on a sequence of the same length as that observed, namely 40 time intervals (39 stages plus the assumption of a decline following the last datum).

To determine this distribution, we generated 10,000 such sequences with a random number generator. We observed the collection of inter-peak lengths for each sequence, calculated a mean and variance of these lengths, and empirically observed the distribution of these means and variances. The mean of the 10,000 sampled means was 3.966, very close to the mean of L . A non-significant one-sixth of the sampled means were less than the mean (3.454) observed in the data. Hence, Hoffman correctly asserted that the mean of the data is not significantly different from the mean expected under his hypothesis. But the mean of the 10,000

sampled variances (3.580) was less than the variance (4) of *L*. This is because rare, very long lengths are eliminated or made less likely by considering only sequences of 40 intervals and because short lengths are made slightly more common by requiring that the sequence end with a decrease.

The central purpose of this note is to determine the significance of the variance estimated from the data. Only 0.07 of the 10,000 sampled variances of inter-peak length under Hoffman's hypothesis were less than 1.19, the variance observed in the data of Raup and Sepkoski¹. Our results verify statistically that the average spacing of peaks is not significantly different from the expected from Hoffman's random process, but show that the variance of spacing is sufficiently small to suggest that Hoffman's non-periodic random process is an inadequate explanation. The observed low variance in the Raup and Sepkoski data string can instead be interpreted to indicate that the pattern is more periodic than Hoffman's random model would imply. Our note is intended to show how statistics can better enable this controversy to be argued on the bases of data and hypotheses.

JENNIFER A. KITCHELL*

GEORGE ESTABROOK†

* Department of Geological Sciences
and Museum of Palaeontology,
University of Michigan, Ann Arbor,

† Division of Biological Sciences,
University of Michigan, Ann Arbor,
Michigan 48109, USA

1. Raup, D. M. & Sepkoski, J. J. Jr *Proc. natn. Acad. Sci. U.S.A.* **81**, 801–805 (1984).

2. Hoffman, A. *Nature* **315**, 659–662 (1985).

HOFFMAN REPLIES—Sepkoski and Raup disagree with my view¹ that 26-Myr periodicity of extinctions is only insufficiently demonstrated and may reflect an effect of stochastic processes. They argue that their more recent, as yet unpublished analyses of the complete, uncultured data set on marine animal families and genera still indicate a significant 26-Myr periodicity. This result is not surprising, given the nature of their statistical test, the definition of extinction events as local maxima, and the geological timescale with approximately constant stage durations. In fact, it is precisely what should be expected if my arguments about the stochastic nature of the pattern of extinctions are correct. Genus-level data do not avoid the biases of the fossil record and the arbitrariness of taxonomic decisions.

Two other predictions made in ref. 1 appear to be confirmed by these more recent analyses. I suggested, first, that the test developed by Raup and Sepkoski² should not reject periodicity even if a few minor peaks are omitted; and second, that 35- to 40-Myr periodicity should appear

in the Palaeozoic. As reported by Jablonski³ and Rampino and Stothers⁴, both these predictions have been confirmed by Raup and Sepkoski's new analyses.

Sepkoski and Raup now contend that my illustration of the variety of geological stages that appear to contain extinction events, depending on definition and timescale, in fact provides strong support for 26-Myr periodicity. They reach this surprising conclusion by identifying the stages which consistently (that is, in more than half of the examples I considered) stand out as peaks of extinction, and by subsequently analysing their distribution according to the timescales of Harland *et al.*⁵ and Odin⁶. This procedure, however, is inappropriate. The majority of these examples are normalized for duration (and are thus strongly timescale-dependent) and each assumes a different timescale. The periodicity can be validly analysed only if the extinction rate is plotted against the timescale for which it was calculated (as in Fig. 1 of ref. 1). Moreover, my illustration assumes two different extinction processes. If the process of family extinction is viewed as possibly continuous rather than necessarily catastrophic, and hence if time normalization of extinction data is required, then the Coniacian and Rhaetian show up as peaks more consistently than the Cenomanian and Norian. The pattern of variation illustrated in ref. 1 does not represent statistical noise but mutually incompatible assumptions. Therefore, its statistical analysis as a single sample is meaningless.

Sepkoski and Raup's contention is also based on factually incorrect premises. Contrary to their assertion, I did illustrate peaks of the number of family extinctions per stage (*B* in Table 1 of ref. 1). One is therefore left with 20 (instead of Sepkoski and Raup's 21) patterns of extinction. The Barremian and Ladinian show peaks as consistently as the Cenomanian and Norian. These two stages—and also the Pliocene, explicitly acknowledged by Sepkoski and Raup as a major event—should also be included in their list of presumed extinction events. Contrary to their other assertion, this list does not include all events recognized by palaeontologists in the late Permian–Quaternary interval. The Toarcian (rather than Pliensbachian) event was identified by Hallam⁷, and Sepkoski⁸ cited additional preliminary evidence. The Callovian (ref. 9 and W.

Brochwiez-Lewinski, personal communication), mid-Oligocene^{10,11}, and of course Pleistocene¹² have also been cited as extinction events. On the other hand, the late Eocene event is strongly contested even as regards the marine plankton¹³, the only group that, according to Sepkoski⁸, experienced at least fairly severe extinction at that time.

Finally, Sepkoski and Raup argue that the pattern of family extinctions deviates significantly from randomness. Their original test, however, involves randomization of the extinction pattern within the constraints of the definition of extinction events as local maxima and the timescale with approximately constant stage durations². That these constraints are not trivial is clearly indicated by the fact that, whether the extinction data were all assigned to the beginnings, the mid-points, or the ends of individual stages, the periodicity was essentially the same². This is possible only with constant stage durations. Otherwise, discordant shifts in the timing of events would affect the pattern of temporal relationships among events. Thus, the random distribution generated by Raup and Sepkoski is inherently biased. It is artificially truncated and its variance is smaller than it would be in a truly random model of the temporal distribution of peaks of extinction. Consequently, it is inadequate for testing my proposed hypothesis of randomness.

It takes only a glance at the pattern of peaks in family extinctions per stage¹⁴ to see that it is not perfectly periodic. Therefore, Gilinsky misses the point of the argument when he calculates the infinitesimally small probability of a random walk yielding a perfectly periodic pattern. Precisely the same mistake was made by Gould¹⁵. (Gilinsky, however, correctly points out that Hoffman and Ghiold's¹⁴ statement about inevitability of "the appearance of approximately 26 million year periodicity" may suggest an incorrect conclusion; we should have emphasized more strongly the words "appearance" and "approximately".) The question to be addressed is not if a perfect periodicity is likely to be produced by random walk, but rather if the approximate periodicity observed in the fossil record is sufficiently close to perfect regularity that it is unlikely to be produced by random walk. A world of difference distinguishes these two questions. I suggest that available statistical tests are insufficiently

1. Hoffman, A. *Nature* **315**, 659–662 (1985).

2. Raup, D. M. & Sepkoski, J. J. Jr *Proc. natn. Acad. Sci. U.S.A.* **81**, 801–805 (1984).

3. Jablonski, D. *Paleobiology* **10**, 139–145 (1984).

4. Rampino, M. R. & Stothers, R. B. in *The Galaxy and the Solar System* (eds Smoluchowski, R., Bahcall, J. N. & Matthews, M. S.) (University of Arizona Press, Tucson, in the press).

5. Harland, W. B. *et al.* *A Geologic Time Scale* (Cambridge University Press, 1982).

6. Odin, G. S. (ed.) *Numerical Dating in Stratigraphy* (Wiley, New York, 1982).

7. Hallam, A. *Paleobiology* **3**, 58–73 (1977).

8. Sepkoski, J. J. Jr *Spec. Pap. geol. Soc. Am.* **190**, 283–289 (1982).

9. Fischer, A. G. & Arthur, M. A. *Spec. Publ. Soc. econ. Paleont. Miner.* **25**, 19–50 (1977).

10. Keller, G. *Mar. Micropaleont.* **7**, 463–486 (1983).

11. Prothero, D. D. *Science* **229**, 550–551 (1985).

12. Martin, P. S. & Klein, R. G. (eds) *Quaternary Extinctions. A Prehistoric Revolution* (University of Arizona Press, Tucson, 1984).

13. Corliss, B. H. *et al.* *Science* **226**, 806–810 (1984).

14. Hoffman, A. & Ghiold, J. *Geol. Mag.* **122**, 1–4, (1985).

15. Gould, S. J. *Discover*, November, 89–91 (1985).

sensitive to reject the hypothesis of randomness in this case.

In fact, Kitchell and Estabrook tested in a rigorous fashion the appropriate null hypothesis. Given their result that 7.9% of 10,000 random walks appear more periodic than the actual pattern of extinctions, one may conclude that my model is not unlikely to provide an adequate explanation for the empirical pattern.

As explicitly stated in my original article, there may be periodicity in late Permian to Quaternary extinctions, but the evidence is as yet insufficient. On the basis of my analysis, which is now confirmed by Kitchell and Estabrook's result, I concluded that I could not reject the hypothesis of randomness of the pattern of extinctions. I suggest that Raup and Sepkoski might have done the same if they had considered the appropriate null hypothesis.

ANTONI HOFFMAN

Lamont-Doherty Geological Observatory,
Columbia University,
Palisades, New York 10964, USA

Endocast morphology of Hadar hominid AL 162-28

ACCORDING to Falk¹, the endocast morphology of the Hadar hominid AL 162-28 is ape-like, contrasting with Holloway's² original conclusion that this specimen exhibits important human-like cortical features. We contend that Falk's interpretation is the result of a major error in her orientation of the endocast.

A comparison of Falk's Fig. 1 (redrawn here as Fig. 1a) with a photograph of the AL 162-28 calvaria in norma lateralis (Fig. 1b) demonstrates Falk's error. In her orientation, the middle arched portion of the squamosal suture, clearly identifiable on the calvaria (SS in Fig. 1b; see also ref. 3), lies below the level of asterion, and the planum occipitale faces almost directly superiorly with the planum muchale almost vertical. Clearly, this combination of features does not characterize any normal hominoid cranium (nor is it found in any other hominid crania from Hadar or elsewhere), and it is apparent that the endocast must be rotated some 40° in a clockwise direction to achieve correct anatomical orientation. When this is done, the cerebellar fossae are tucked under (and are rostral to) the occipital poles, as in all hominids and almost all apes. Falk⁴ has defended her orientation by citing Kimbel³ to the effect that the cerebellar fossae are deeper than the cerebral fossae. Correcting the specimen's orientation, the cerebellar fossae are deeper, but certainly not more posteriorly projecting, a position Kimbel never claimed.

Figure 1 indicates Falk's identification of the cortical features on the endocast.

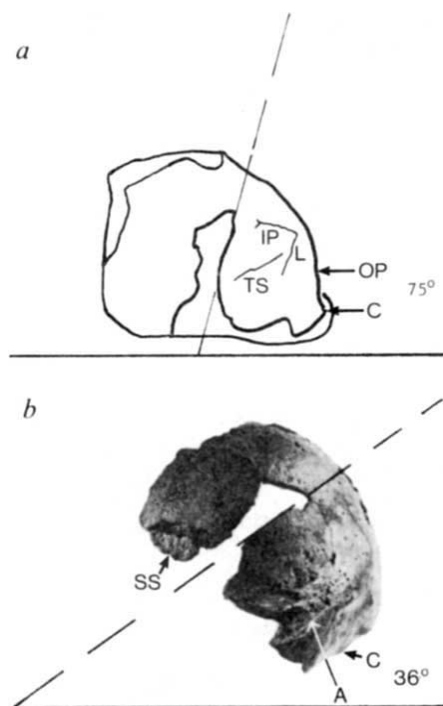


Fig. 1 Diagrams showing the error in Falk's¹ orientation of the Hadar AL 162-28 endocast. *a*, A tracing from Falk's Fig. 1; *b*, a lateral view of the AL 162-28 original cranial fragment correctly oriented. The difference between the two orientations is approximately 39° as measured from the dashed lines intersecting the horizontal planes, using the notch created by the missing bone as a landmark. Falk rotated the endocast 39° in an anticlockwise direction, thereby erroneously positioning the cerebellar lobes posterior to the occipital poles of the cerebrum. SS, squamosal suture; A, asterion; C, cerebellum; OP, occipital pole; IP, interparietal sulcus; TS, the superior temporal sulcus, and L, the lunate sulcus are Falk's designations¹.

The rostral position of the lunate sulcus (L in Fig. 1a) is said to be an ape-like condition. When the endocast is correctly oriented, however, L (as identified by Falk) crosses the posterior aspect of the endocast in a more caudal position than on ape brains⁵. The relative caudal location of Falk's L is confirmed by its close proximity to the occipital pole (OP); the distance between L and OP on the AL 162-28 is approximately half that separating these structures in a sample of 10 chimpanzee brain casts (the hominid falls 5.5 s.d. below the ape mean), despite the fact that eight of these brain casts have volumes less than the 375–400 cm³ estimated for the Hadar endocast, including one chimpanzee infant. (See data in ref. 6, in which the distance from the interparietal sulcus (IP) to OP is the same as the OP–L distance.)

The lambdoidal suture is discernible on the internal aspect of the Hadar calvaria³, where, on both sides, it is 2 mm (not 5 mm, as claimed by Falk¹) below the feature

corresponding to Falk's L. We suggest that Falk's L may be a manifestation of the lambdoidal sutural complex which is frequently reproduced on chimpanzee endocasts as a distinct furrow (feature 'x' in ref. 7). Holloway^{2,6} recognized the likelihood that feature 'x' masks the position of L on the AL 162-28 endocast. We do not, therefore, believe that L can be identified unequivocally on this specimen. But if Falk is correct in her identification of the lunate sulcus, and does accept Holloway's² identification of the interparietal sulcus, then both the correct orientation of the specimen and metric data⁶ confirm that this structure occupies a caudal position on AL 162-28, a major derived hominid condition.

RALPH L. HOLLOWAY

Department of Anthropology,
Columbia University,
New York, New York 10027, USA

WILLIAM H. KIMBEL

Institute of Human Origins,
2453 Ridge Road,
Berkeley, California 94709, USA

1. Falk, D. *Nature* 313, 45–47 (1985).
2. Holloway, R. L. *Nature* 303, 420–422 (1983).
3. Kimbel, W. H., Johanson, D. C. & Coppens, Y. *Am. J. phys. Anthropol.* 57, 453–499 (1982).
4. Bower, B. *Sci. News* 127, 157 (1985).
5. Connolly, C. J. *External Morphology of the Primate Brain* (Thomas, Illinois, 1950).
6. Holloway, R. L. in *Hominid Evolution: Past, Present and Future* (ed. Tobias, P. V.) 47–64 (Liss, New York, 1985).
7. Le Gros, Clark, W. E., Cooper, D. M. & Zuckerman, S. *J. R. anthrop. Inst.* 66, 249–268 (1936).

FALK REPLIES—Holloway and Kimbel claim that my interpretation of the sulcal pattern of AL 162-28 is due to an error in the orientation of the endocast because “in her orientation, the middle, arched portion of the squamosal suture, clearly identifiable on the calvaria . . . lies below the level of asterion . . .”. Kimbel *et al.*'s photograph of the left side of the calvaria shows neither the apex of the squamosal suture nor the external (temporal bone) portion of that suture¹. What it does show is a 13.5 segment of the temporal margin of the parietal bone that is, by their own description, *posterior* to (where) the apex of the squamosal suture (would have been). Furthermore, because of overlap at the squamosal suture, the temporal margin of the parietal bone which is present in AL 162-28 would have been *below* the external (temporal bone) portion of the squamosal suture. Unfortunately, “a long, narrow strip of external table is lost just above the left parietal's temporal margin”¹. Neither the squamosal suture nor asterion is reproduced on the endocast, and I do not think the calvaria of AL 162-28 is complete enough to allow speculation about where the locations and relative positions of both features would have been on the (whole) endocast. Even if Holloway and Kimbel's SS and A identifications (Fig. 1b above) could be

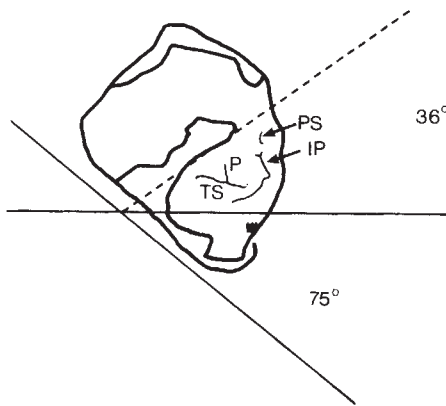


Fig. 2 Holloway and Kimbel's Fig. 1a rotated to correspond to their preferred orientation. Such an orientation for the sulci on AL 162-28 is unlike that seen in other primates. On the other hand, the configuration of these sulci oriented as in Fig. 1a is common for primates. PS, superior parietal sulcus; P, superior parallel branch of TS; other abbreviations as for Fig. 1a.

interpreted and projected onto the endocranium as they suggest, given the overlap at the squamosal suture and the variation of its course in hominoids, there is ample room for forward rotation of their calvaria within the normal hominoid range. (Walker *et al.*² describe a hominoid cranium with a vertically oriented planum occipitale, which is what I believe Holloway and Kimbel meant by the planum occipitale facing almost "directly superiorly".)

More to the point, I have rotated Holloway and Kimbel's Fig. 1a to correspond to their preferred orientation, and traced the sulci back on to my original³ Fig. 1 (see Fig. 2). The angle of the intraparietal sulcus (IP, an identification on which Holloway and I agree) is nearly vertical, whereas the angle of the superior temporal sulcus (TS, not mentioned by Holloway and Kimbel) is nearly horizontal. This combination of sulci does not occur in this orientation for primates⁴, but this combination of sulci in the orientation shown in Fig. 1a is common for primates⁴. The question of orientation of sulci is not to be dismissed lightly, since Radinsky⁵ has shown that the issue of direction and orientation of sulci is fundamentally important for primate brain evolution (especially the origin of anthropoids).

If I am correct in my observations, then Holloway and Kimbel's statistics on distances between points on the endocranium are not useful since all of them would depend on an incorrectly identified occipital pole (OP) (if one is used which is consistent with Holloway and Kimbel's orientation of the endocranium). The OP which I have identified, on the other hand, does not project as far caudally in this specimen as it does in most hominoids. This alone

would account for shortened distances. Furthermore, absolute distances should not be used to refute a chimpanzee-like sulcal pattern; ratios would be better.

Holloway and Kimbel contend that the feature I identify as the lunate sulcus (L) may be a groove caused by lipping at the lambdoid suture, and they cite LeGros Clark's⁶ study of six chimpanzee brains and corresponding endocrania to support their contention. I have shown that the course of the lambdoid suture (determined from its traces and their relationship to ridge X on the endocranium that corresponds to the nuchal crest) runs 5 mm caudal to the feature I identify as L on AL 162-28 (Fig. 1 of ref. 3). Holloway and Kimbel say (above) that the lambdoid suture is 2 mm rather than 5 mm below the feature I identify as L. I have repeated my analysis and again find the distance to be 5 mm. How can this feature be due to lipping of a suture that is 5 mm behind it? Furthermore, how can Holloway and Kimbel attribute a 'groove' on an endocranium to a skull suture which is so smooth and obliterated that it can be traced only with difficulty¹?

Both the lunate sulcus and a groove due to the lambdoid suture may be reproduced on endocrania of chimpanzees⁶ and presumably on those from early hominids. How are we to decide which of these two features is the disputed feature on endocranium AL 162-28? As noted above, the feature in question cannot be due to lipping at the lambdoid suture because that suture is 5 mm away and because the suture does not lip in this specimen, that is, it is so smooth it is almost obliterated.

On the other hand, the feature in question merges with the intraparietal sulcus (IP in Fig. 2), as does the lunate sulcus in *Pan*; it is rostral to the lambdoid suture, as is the lunate sulcus in *Pan*; and has a sulcus-like texture and shape similar to the lunate sulcus in *Pan*. I therefore believe that the feature in question on AL 162-28 is a lunate sulcus that is similar to those seen in brains of chimpanzees, a finding that is consistent with the entire sulcal pattern that is reproduced on the endocranium of AL 162-28.

DEAN FALK

Department of Anatomy/Caribbean
Primate Research Center,
University of Puerto Rico
School of Medicine,
PO Box 5067, San Juan,
Puerto Rico 00936

1. Kimbel, W. H., Johanson, D. C. & Coppens, Y. *Am. J. phys. Anthropol.* **57**, 453-499 (1982).
2. Walker, A., Falk, D., Smith, R. & Pickford, M. *Nature* **305**, 525-527 (1983).
3. Falk, D. *Nature* **313**, 45-47 (1985).
4. Connolly, C. J. *External Morphology of the Primate Brain* (Thomas, Illinois, 1950).
5. Radinsky, L. B. *Am. Sci.* **63**, 656-663 (1975).
6. Le Gros Clark, N. E., Cooper, D. M. & Zuckerman, S. J. *R. anthrop. Inst.* **66**, 249-268 (1936).

Maternal investment in mammals

IN a recent paper Martin and MacLarnon¹ claim to clarify the allometry of neonatal size and gestation period of mammals, attributing particular importance to the distinction between altricial and precocial taxa. In addition, Martin and MacLarnon suggest that maternal investment, as reflected by a fetal growth parameter, is higher for altricial than precocial mammals. Unfortunately, we find that such conclusions are not warranted and propose that other interpretations are possible.

We are confused by Martin and MacLarnon's definition of maternal investment. They suggest that altricial mammals have high maternal investment indicated by high fetal growth "factors", with precocial mammals having the opposite traits. Yet, in mammals bearing altricial young, overall maternal investment at birth actually is low: gestation periods are relatively short^{1,2} and neonatal weights are typically low¹. Hence, it confuses the issue to say that high fetal growth rates are indicative of high maternal investment. Using different measures of parental investment is revealing³. However, we maintain that the neonate's developmental status and the time and energy put into it are the most important criteria in evaluating maternal investment at birth.

Also, we cannot accept their definitions of altricial and precocial: precocial mammals are those with a litter size less than 1.5 and eyes open at birth; altricial mammals have litter sizes equal to or greater than 3, and the eyes are closed at birth. Taxa that do not conform to either of these criteria are placed in an "intermediate" group. Even humans, which are highly altricial⁴, would be classified as precocial by such standards. We² and others⁵ have experienced difficulty classifying neonates as altricial versus precocial because these categories are discrete and arbitrary, whereas in reality mammalian species occur along a continuum of developmental maturity at birth. In a previous study of the relationship between maternal investment at birth and mating systems in mammals, we at first used definitions from the literature to classify a species as altricial or precocial. However, because of problems with a simplistic 'either/or' categorization scheme, we subsequently used several relative measures of maternal investment at birth instead². One of these measures, neonate weight/litter size standardized for body weight, is useful as it describes investment in an individual neonate relative to the number of siblings it shares that investment with. Indeed, we found that 78% of mammals with litter sizes of ≤ 2 bear precocial young and 87% of those with at least 3 young have altricial offspring. Yet, this does not imply that

litter size should be a criterion for classification of neonate development.

Classifying species as altricial versus precocial based on differences in litter size introduces a serious confounding effect not discussed by Martin and MacLarnon¹. In particular, we note that fetal growth parameters for precocial taxa are essentially standardized for constant litter size as most of these taxa have only one young per litter (by Martin and MacLarnon's definition). By contrast, we find a great variability in litter size among altricial species (again, using their definition). That litter size is inversely correlated with adult body size among mammalian species⁶⁻⁸ confounds Martin and MacLarnon's analysis of how fetal growth relative to body size differs between altricial and precocial species. A larger litter size results in each individual offspring obtaining a relatively smaller fraction of the mother's total parental investment at birth⁹. Growth rates of such offspring might be comparatively high simply to increase survivorship.

If a more rigorous consideration of neonatal status is used²⁻⁴, the discreteness of altricial and precocial taxa is not as great as suggested by Fig. 1 of ref. 1. Related to this, we find the legend to Fig. 2 inappropriate because it is not obvious that an "overall best-fit line of fixed slope 0.1" essentially constitutes a linear discriminant function between the 'altricial' and 'precocial' taxa. Their discriminant function with a 0.1 slope is as effective with a slope of 0; there are about five misclassified points using either slope. This suggests that Martin and MacLarnon's altricial and precocial categories are more explicitly defined as groups of short versus long gestation period. Realization of this leads us to question seriously the relevance of Martin and MacLarnon's use of this 0.1 slope as an allometric coefficient, as in their equations (6) and (8)¹, since it has no biological basis.

Allometric pattern is often presumed to reflect physiological constraints to life history alternatives. Although we recognize that such constraints certainly exist, we caution that sampling bias might be present because the representation of mammals of various body sizes is seldom independent of environmental variation. Large mammal species are most numerous in regions of pronounced seasonality¹⁰. We suggest that natural selection may favour rapid growth rates in highly seasonal environments to ensure that the young attain adequate size during the limited growth season to enhance survival through the oncoming winter or seasonal drought. This implies that environmental seasonality selects for a suite of life-history attributes which increase survival during periods of resource shortage, but which capitalize on abundant resources during the growth season^{11,12}. Such a sampling bias may well account for the positive

correlation between "fetal growth factor" and body mass as described by Martin and MacLarnon¹.

SAMUEL I. ZEVELOFF

Department of Zoology,
Weber State College,
Ogden, Utah 84408, USA

MARK S. BOYCE

Department of Zoology and Physiology,
University of Wyoming,
Laramie, Wyoming 82071, USA

1. Martin, R. D. & MacLarnon, A. M. *Nature* **313**, 220-223 (1985).
2. Zeveloff, S. I. & Boyce, M. S. *Evolution* **34**, 973-982 (1980).
3. Knapton, R. W. *Can. J. Zool.* **62**, 2673-2674 (1984).
4. Zeveloff, S. I. & Boyce, M. S. *Am. Nat.* **120**, 537-542 (1982).
5. Case, T. J. *Q. Rev. Biol.* **53**, 243-282 (1978).
6. Tuomi, J. *Oecologia* **45**, 39-44 (1980).
7. Eisenberg, J. F. *The Mammalian Radiations* (University of Chicago Press, 1981).
8. Millar, J. S. & Zammuto, R. M. *Ecology* **64**, 631-635 (1983).
9. Smith, C. C. & Fretwell, S. *Am. Nat.* **108**, 499-506 (1974).
10. Zeveloff, S. I. thesis, Univ. Wyoming, Laramie (1982).
11. Boyce, M. S. *Am. Nat.* **114**, 569-583 (1979).
12. Lindstedt, S. L. & Boyce, M. S. *Am. Nat.* **125**, 873-878 (1985).

MARTIN AND MACLARNON REPLY—The points raised by Zeveloff and Boyce indicate the need for further clarification of issues raised by our paper¹, and we are glad of the opportunity to comply.

There is, indeed, potential confusion surrounding the definition of 'maternal investment'. In our paper, we implicitly used this term in relation to the mother's investment per unit time in the growth of an individual fetus. In order to convert this to total investment per unit time during pregnancy, it would, of course, be necessary to multiply each fetal growth factor by litter size for the species. Thus, the distinction between altricial and precocial mammals in this respect is far more marked than indicated in our Fig. 3. However, we recognize that maternal investment could be defined as total investment in individual neonates, regardless of the time over which the investment is spread. By such a definition, mothers clearly invest more in precocial neonates than in altricial neonates. We would defend our definition on the grounds that it relates to the mother's division of her total resources (which are heavily influenced by her body size) between reproduction and other functions during pregnancy. Clearly, accurate calculation of total maternal investment by any definition would require data on lifetime reproductive performance.

The double criterion that we used to define altricial as opposed to precocial mammals (combining time of eye opening and litter size) was a quantitative translation of two features originally emphasized by Portmann² in his pioneering studies separating 'Nesthocker' from 'Nestfluchter' in both birds and mammals. While we accept that some gradation exists (and, in fact, we allowed for it in our paper—

intermediates), the important point is that a clear bimodal distribution among mammals emerges when gestation period is considered in relation to body size, closely matching the distinction between altricial and precocial categories. Incidentally, although human infants are often described as 'altricial', they are precocial both by Portmann's definition and by ours. It has been shown that the relative helplessness of the human neonate is a special condition, unique among mammals, related to an unusual pattern of postnatal brain development^{3,4}.

The fixed slope value of 0.1 used to derive the histogram in our Fig. 2 was initially determined by the average empirical value for major mammalian groups with a single neonate type (our Table 1). The iterative technique reported was used as an additional approach, primarily to investigate the distributions of residuals generated with different slope values. As Zeveloff and Boyce point out, altricial and precocial mammals (as defined by us) are to a large extent separated by gestation period even without taking maternal body size into account (that is, slope value of 0). This, surely, is one of the most convincing indications that there is an underlying split between altricial and precocial mammals. The histogram of residuals in fact becomes trimodal when a slope value of 0 is used for all gestation periods, as certain large-bodied altricial mammals (for example, some carnivores) overlap with certain small-bodied precocial mammals (for example, some primates, and some hysticomorph rodents). This overlap is largely eliminated when a slope value of 0.1 is used, and an order-by-order analysis of residuals (unpublished) shows that the resulting pattern is entirely in accord with Portmann's interpretation of the altricial/precocial division.

As Zeveloff and Boyce themselves produce a separation of mammals into two groups that are 80% in agreement with our altricial/precocial division, despite an entirely different approach, there would seem to be an underlying biological reality in such distinctions, regardless of any disagreement about finer details. It is important that debate about the details should not obscure this fact.

R. D. MARTIN

A. M. MACLARNON

Department of Anthropology,
University College London,
Gower Street,
London WC1E 6BT, UK

1. Martin, R. D. & MacLarnon, A. M. *Nature* **313**, 220-223 (1985).
2. Portmann, A. *Biomorphosis* **1**, 109-126 (1938).
3. Portmann, A. *Revue suisse Zool.* **48**, 511-518 (1941).
4. Martin, R. D. *Human Brain Evolution in an Ecological Context* (American Museum of Natural History, New York, 1983).

Molecular dynamics resolved

from David D. Thomas

Time-resolved optical and magnetic resonance techniques offer complementary views of large-scale cooperative molecular motions within macromolecular assemblies.

ONE of the most exciting trends in biophysical chemistry is the increasing emphasis on understanding, quantitatively, the role of molecular motions in biological function. While much of the study of molecular dynamics has focused on small-scale intramolecular motions on the picosecond or nanosecond time scale, it is increasingly apparent that the large-scale motions within assemblies such as membranes and chromosomes are crucial to the understanding of cellular biophysics. This article presents an overview of recent advances in the technology for probing rotational molecular motions in the microsecond time range. The discussion will address the two most effective techniques: transient optical anisotropy and saturation transfer electron paramagnetic resonance (ST-EPR).

Both of these techniques rely on the use of extrinsic spectroscopic probes with excited-state lifetimes in the microsecond to millisecond time range¹. The optical probes are mainly derivatives of eosin or erythrosin, organic dyes that have efficiently populated and stable triplet states. The paramagnetic probes are nitroxide spin labels.

Each experiment begins with a pulse of exciting radiation that causes a transient decrease in the number of ground-state probes available for subsequent excitation. This pulse of radiation is delivered so that it selectively excites molecules with a particular orientation in space ('photo-selection'), and the sample's absorption or emission is monitored with the same orientational preference. The system re-equilibrates as excited probes return to the ground state; the recovery is accelerated by rotational motion that brings un-

excited molecules into the detectable orientation range. Of course, rotational motion is detectable only if it occurs within a time comparable to or less than the excited-state lifetime of the probe — hence the need for μ s lifetimes in the study of μ s motions.

Optical anisotropy

An example of the optical data for transient absorption anisotropy (TAA) is shown in Fig. 1. The instrument used in this experiment has evolved from those designed by R. Cherry and others in the 1970s²⁻⁴. In this case, a nitrogen-pumped dye laser excites the sample with an intense vertically polarized 5-ns pulse, and a low-intensity beam from an argon-ion laser is used to monitor the time-resolved recovery of the polarized absorption following the pulse. The fast transient digitizer (LeCroy 2256, 50 ns/time point) permits high time-resolution, while the high-repetition-rate (200 Hz) pulsed laser (Lambda-Physik EMG-53MSC), along with a microcomputer-controlled direct-memory-access (DMA) circuit (LeCroy 3500) permits very rapid data acquisition and signal averaging, minimizing the time required for high-quality data acquisition. Although this particular configuration was constructed of commercially available components^{1,5}, an instrument that approaches some of its capabilities is available from PRA (London, Ontario).

The function plotted in Fig. 1 is the absorption polarization anisotropy, whose exponential decay constants (rotational correlation times) and amplitudes are related directly to the time constants and amplitudes of molecular rotational motions^{1,5,6}. Similar experiments probing

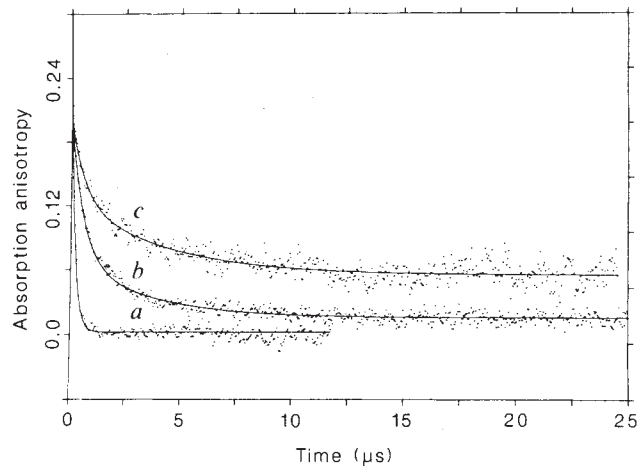


Fig. 1. Transient saturation transfer EPR (saturation recovery) data from maleimide-spin-labelling haemoglobin solutions corresponding to the rotational correlation times τ . The bottom trace in each case is the motion independent recovery, governed by T_1 , and the top trace shows the increase in the recovery rate when the effects of rotational motion are included⁸.

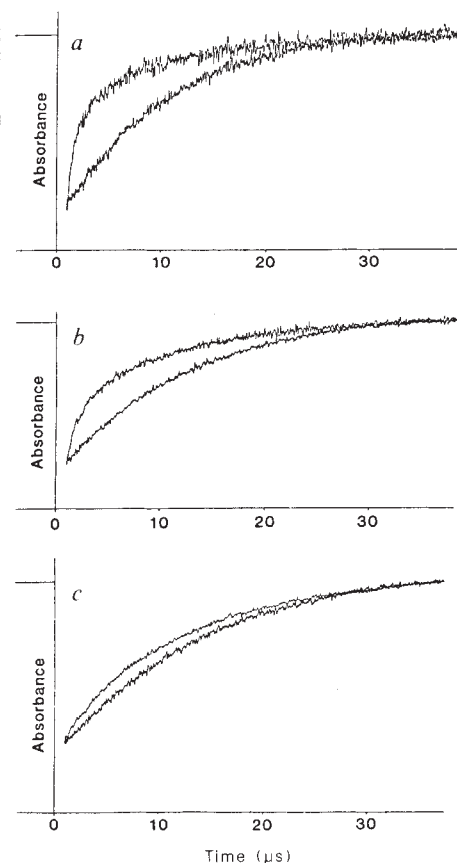


Fig. 2. Transient absorption anisotropy data from eosin-iodoacetamide attached to myosin heads in (a) the proteolytically isolated head (S-1), (b) the intact myosin monomer, and (c) myosin filaments (several hundred myosin molecules self-assembled essentially as in a muscle fibre)⁷. Note that the rate and amplitude of decay are much less in the filaments, indicating that the microsecond segmental protein motions have been restricted in both rate and amplitude.

emission instead of absorption do not usually achieve the same high time resolution because the laser pulse interferes with detection. But emission studies offer sensitivity sufficient to study extremely small samples, possibly even single cells^{3,4}.

Saturation recovery EPR

As in optical spectroscopy applications, EPR experiments involve transient absorption, which is often referred to in magnetic resonance as saturation recovery or as pulsed saturation transfer EPR, since rotation transfers saturation away from the irradiated spins.

In EPR the exciting radiation is a microwave pulse from a klystron. Photoselec-

tion arises because spin labels with different orientations absorb at different frequencies^{1,7}. This selectivity is more specific than that conferred by optical spectroscopy. Because EPR is an inherently weaker effect than optical absorption, rapid data acquisition and signal averaging (at thousands of transients averaged per second) are even more important⁸. A key element in this instrument is a loop-gap resonator, a device that facilitates the use of intense microwave pulses in EPR spectroscopy⁹.

A pulsed EPR spectrometer⁸ with increased sensitivity was recently developed which expands the range of biological samples that can be studied. Many of the components of this instrument are now commercially available, but commercial systems (from IBM Instruments/Bruker) still fall considerably short in terms of sensitivity and signal averaging rate. Figure 2 illustrates the sensitivity of pulsed ST-EPR to microsecond rotational motion¹⁰. When the motion is slow on the microsecond time scale (Fig. 2c) it has very little effect on the absorption recovery, which occurs with a time constant equal to the relaxation time (excited-state lifetime) T_1 . However, sub-millisecond correlation times result in dramatic increases in rate of recovery (Fig. 2a,b).

In both EPR and optical spectroscopy, time resolution increases the range of motions and systems that can be studied and decreases the ambiguity of data interpretation. While optical spectroscopy has better absolute sensitivity (and can therefore be used to study smaller amounts of material), EPR offers the advantage of orientational resolution¹¹. Thus, a pulsed EPR experiment on a macroscopically oriented system (for example, parallel muscle fibres or stacked membranes) offers the potential for unequalled resolution and accuracy in the elucidation of large-scale motions involved in the function of biological assemblies.

David D. Thomas is in the Department of Biochemistry, University of Minnesota Medical School, Minneapolis, MN 55455 USA.

1. Thomas, D.D. in *Techniques for Analysis of Membrane Proteins* (eds Cherry, R. & Ragan, I.) Ch. 13 (Chapman & Hall, London, 1986).
2. Cherry, R.J. in *Membranes and Transport* (ed. Martonosi, A.) 145-152 (Plenum, New York, 1982).
3. Corin, A.F., Matayoshi, E.D. & Jovin, T.M. in *Spectroscopy and the Dynamics of Biological Systems* (eds Bayley, P.M. & Dale, R.E.) 53-78 (Academic, London, 1985).
4. Garland, P.B. & Johnson, P. in *Spectroscopy and the Dynamics of Biological Systems* (eds Bayley, P.M. & Dale, R.E.) 95-118 (Academic, London, 1985).
5. Eads, T.M., Thomas, D.D. & Austin, R.A. *J. molec. Biol.* **179**, 55-81 (1984).
6. Kinoshita, K.Jr., Kawato, S. & Ikegami, A. *Adv. Biophys.* **17**, 147-203 (1984).
7. Thomas, D.D. *et al.* in *Spectroscopy and the Dynamics of Biological Systems* (eds Bayley, P.M. & Dale, R.E.) 239-257 Academic, London, 1985.
8. Hyde, J.S., Froncisz, W. & Mottley, C. *Chem. phys. Lett.* **110**, 621-625 (1984).
9. Froncisz, W. & Hyde, J.S. *J. Magn. Reson.* **47**, 515-521 (1982).
10. Fajer, P., Thomas, D.D., Feix, J.B. & Hyde, J.S. *Biophys. J.* (submitted).
11. Cooke, R., Crowder, M.S. & Thomas, D.D. *Nature* **300**, 776-778 (1982).

Biochemists converge at the capital

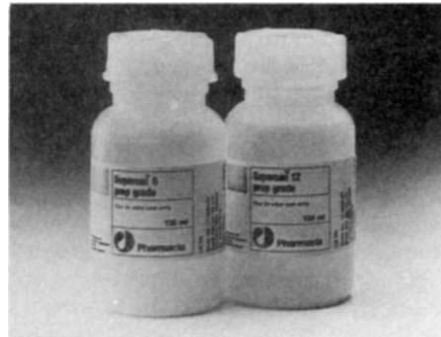
Washington, DC is the host city for the ASBC meeting this year. Almost 200 exhibitors will be competing for the attention of conference visitors.

TIME-resolved **fluorescence detection** can reveal the dynamics of molecules kept in solution or embedded in membranes and proteins. The GREG 200 from ISS Inc. is a multi-frequency cross-correlation phase and modulation fluorometer with the capability to perform such analyses (Reader Service No. 100). Displayed at booth B32, the instrument uses lifetime measurement ranges from a few picoseconds to one millisecond to unravel complex decay processes. With the aid of a dedicated PC, ISS's fluorometer can solve multi-exponential and non-exponential decays; phase- and modulation-resolved spectra allow the separation of spectral components from a mixture of several emitting species with overlapping bands. The GREG 200 will also provide time-resolved spectra in three dimensions, with time along the z axis. ISS can say more about their machine at their ASBC booth.

Pharmacia have filled out their chromatography products with two more additions to the family. The MinoRPC S 5/20 is a **reversed-phase column** designed for the gradient or isocratic separation of small biomolecules, including amino acids and nucleotides (Reader Service No. 101). The bonded phase affords selective recovery of such molecules due to an end-capping process which, Pharmacia claims, also makes column results highly reproducible.

Two preparative-grade matrices for **high performance gel filtration** are also

being offered by Pharmacia to scale-up their prepacked Superose 6 HR 10/30 and 12 HR 10/30 columns (Reader Service No. 102). The new, rigid Superose 6 and



Pharmacia's scale-up matrices.

Superose 12 prep grades are based on 30 μ m agarose beads, stable from pH 2 to 14. With Pharmacia's scale-up grades, preparative separations can be achieved in a few hours. The Pharmacia divisions represented at ASBC will be in the area of booths E46 - F51.

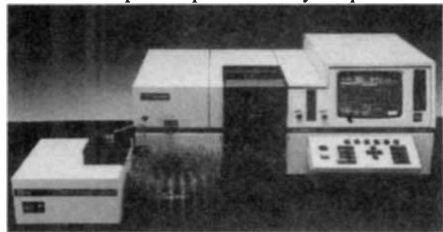
Combining reversed-phase gradient chromatography and detection modules, Waters has created a **gel permeation chromatography** (GPC) system with isocratic or gradient operation, programmable UV detection and stable refractive index (RI) detection (Reader Service No. 103). With the Waters 600 gradient module, the GPC system can analyse additives and low-molecular-weight polymers by gradient RPC, then perform molecular weight determinations for larger polymers by GPC. The 600's flow rate-



RPC and GPC: the dynamic duo in Waters' new system.

control is accurate to ± 0.5 per cent, and its gradient composition will conform to specifications within ± 0.15 per cent. The two detectors in the GPC III system are the Waters 490, which can optimize spectroscopic information for analyses between 190 and 600 nm, and the 410, which carries out refractive index detection. As an option to their new machine, Waters has the 840 data and chromatography control station with Expert software. The package will provide several types of calibrations, processing and storage of GPC data. Waters are showing in the area between booths G46 and H51.

Varian, at ASBC in booths G63 through G70, have recently released several spectrophotometry products



Varian's economical alternative for AA.

including a lower-cost version of their SpectrAA 30/40 series **atomic absorption spectrophotometers**. The new SpectrAA 10 single-beam and the double-beam SpectrAA 20 have retained several automation features of the earlier models, such as centralized control for the automatic sample changer and the GTA-96 graphite furnace (Reader Service No. 104). But the SpectrAA 10/20 series machines can sell for as much as £10,000 (UK) less than the 30/40 series counterparts. A single keyboard ties in to all system components and allows access to the measurement and system control software. Method development and instrument set-up procedures can be stored on the instrument's floppy disk.

Plug-in modules for Varian's latest **UV-vis spectrometer** will make the machine more flexible for individual



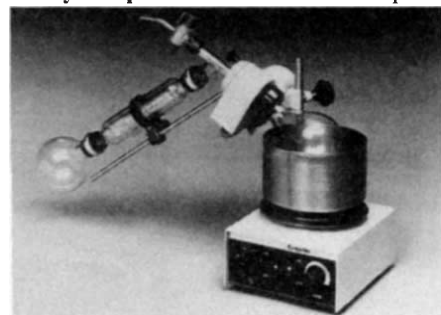
The DMS 200, with more modules in store.

analytical needs (Reader Service No. 105). The first such module offered has a soft-key menu that enables the user to construct data and mathematical routines for calculating final results from raw data. This so-called scan application module includes built-in functions such as spectral subtraction/addition/division, absorbance ratio, peak area and multi-wavelength and dilution correction calculations; stored instrument parameters can be linked with calculation routines to create methods for specific applications. The £8,000 (UK) DMS 200 can then present final results on a high-resolution plotter.

A **compact scintillation counter** from Integrated Genetics fills the niche between low-cost survey meters and high-volume counting instruments that can cost up to \$35,000. The Gene-Trak beta detector directly measures beta particle emissions from samples on membrane filters (Reader Service No. 106). The system was designed to complement Integrated Genetics's *Salmonella* hybridization assay, but many researchers working with DNA and ^{32}P may find the Gene-Trak works well in their experimental regime. Gene-Trak's maker says its counting efficiency compares with that of large-scale instruments, yet their instrument costs less to purchase and operate. Integrated Genetics sells Gene-Trak at \$3,600 (US).

Biochemical side-kicks

For small-scale evaporation, reflux or off-distillation, Haake Buchler makes a **micro rotary evaporator** with variable speed



Small-scale evaporation with a twist.

between 80 and 200 r.p.m. (Reader Service No. 107). The adjustable speed allows effective evaporation of viscous liquids; an inlet tube which feeds to the evaporation flask removes the need for operator intervention. Haake Buchler's evaporator comes with a stainless steel bath and complete set of glassware (evaporating flasks in 50, 100 and 250 ml sizes, with corresponding receiving flasks) for less than \$1,200 (US). The entire unit measures 200 mm width $\times$ 235 mm depth $\times$ 165 mm height. At ASBC, Haake Buchler will have more information in booths G16 through G20 even.

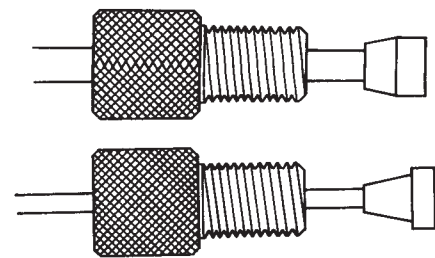
Two equipment companies have recently tackled problems with Teflon tub-



Customized lumen configurations from Zeus.

ing. Zeus Industrial Products invites custom designs for multi-lumen sub-miniature tubing (Reader Service No. 108). The company can manufacture **Teflon tubing** with two or more constant-diameter, continuous-length passages in a range of internal and outer diameters. Such tubing is flexible, impervious to most corrosives, inert, non-toxic and heat-resistant up to 500°F. Zeus's prices vary for standard ready-made and customized tubing.

Upchurch Scientific have developed flangeless fittings for Teflon and plastic



Ferrules replace flanging.

tubing that can be directly substituted for all flange-type fittings (Reader Service No. 109). These fittings consist of a Tefzel ferrule, which slides onto the end of the tubing, and a Delrin male nut which must be finger-tightened. Upchurch's fittings are compatible with 1/4-28 or metric threads. The ferrule for 1/16-inch tubing holds to over 1,000 p.s.i., and the 1/8-inch ferrule is good for over 500 p.s.i., according to Upchurch. The fittings go for less than \$4 (US) each.

Another brand of **acrylamide** has become available to biochemists; this one, from Serva Fine Biochemicals (Reader Service No. 110). Serva maximize their product's stability by packaging it in nitrogen and storing it at -20°C . Servalyt Precotes, Serva's 0.1 mm precast gels, are made from the same acrylamide, which Serva is now offering for home-made gels at \$27 (US) per 100 g bottle.

Hamilton's PB600 **dispenser** can be fitted with syringes of different sizes to yield repeated volumes, each 2 per cent of the syringe capacity (Reader Service No. 111). Depending on syringe size, the instrument's range of volumes extends between 0.5 μl and 200 μl . Fitted with

Hamilton's LT syringes, the dispenser-syringe unit is autoclavable at 121°C; alternatively, the dispenser can be attached to an adaptor that enables standard disposable tips to be used. Hamilton will be located at booth G23 at ASBC to answer questions on this and other products.

Bench-top varieties

An updated version of the Eppendorf **microcentrifuge** came from Brinkmann this year. The new model 5415 can ac-



Eppendorf expands its accommodations.

commodate up to eighteen 1.5-ml microcentrifuge tubes, or accept adaptors which expand its capabilities to 250, 400 and 500 µl tubes (Reader Service No. 112). The Eppendorf rotor holds tubes at a 45° angle, the ideal inclination for pellet formation. The rotor is also enclosed to reduce air turbulence and noise. Brinkmann's new Eppendorf costs \$1,295 (US) and can be operated in a cold room. Its speed tops out a 14,000 r.p.m., and timing, as well as speed, is adjustable. Brinkmann will be in booths E15 through E19 (odd) at ASBC.

In scores of booths between D4 and F11, Beckman Instruments will be exhibiting their wares, one of which is the TL-100 **table-top ultracentrifuge**. Beckman has brought out another rotor for the \$19,590 (US) machine, itself just introduced this spring. The fixed-angle TLA-100.3 rotor spins larger sample volumes than previous varieties could accommodate, in 3 ml open-top or 3.5 ml sealed tubes (Reader Service No. 113). Six tubes at a time can be spun at speeds up to 541,000 g. Beckman's TLA-100.3, with a k factor of 16.5 at top speed, sells for \$2,970 (US). With the other rotors available, the TL-100



Beckman's ultracentrifuge: smaller, but still speedy.

allows ultracentrifugation of samples as small as 0.2 ml, and can process samples up to four times faster than floor models, says Beckman. It has a controlled temperature environment ideal for applications such as rapid sample clarification, density gradient separation, isolation of plasmid DNA, molecular weight determinations and binding studies.

A **compact bench-top centrifuge** from Jouan will centrifuge up to 48 5 ml tubes at 1,630g. The B311, designed for laboratories that do not require high carrying capacities, features closed loop speed control, digital speed readout, dynamic electric braking and an imbalance detection and shutdown system (Reader Service No. 114). In addition, the B311 has

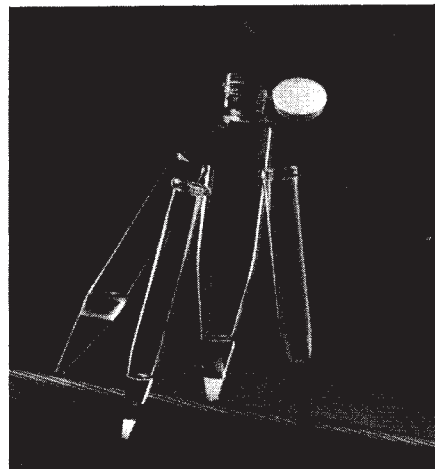


Flexible arrangements for Jouan's benchtop.

several adaptors and options that broaden its usefulness without sacrificing its \$1,500 (US) base price. A swing bucket rotor carries tubes ranging from 1.5 ml microtubes through 180 ml bottles. Fifteen and 50 ml conical tubes can also be centrifuged using a different carrier, and aerosol containment covers are available for toxic or pathogenic samples. Jouan will have booths at J37 and J39 in Washington.

Kimble now manufactures a selection of plain-top and 50 ml screw-top **disposable glass centrifuge tubes** (Reader Service No. 115). The tubes come in 5, 10, 15 and 50 ml sizes, and all but the 50 ml are available with polyethylene snap caps. In

screw-thread sizes, the new 50 ml complements an existing array of disposable tubes. These tubes are topped by black phenolic caps with rubber or PTFE/rubber liners. All of Kimble's disposable centrifuge tubes are made from borosilicate glass that resists organic solvents and preserves pH-sensitive solutions. Kimble

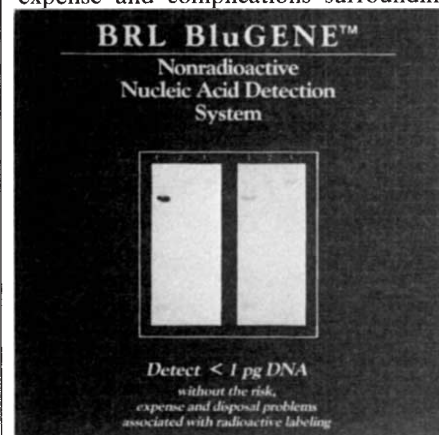


Kimble's toss-away tubes.

protects its product from contamination in shrink-wrapped low particle trays; costs vary depending on size and quantity.

Away from radiation

Bethesda Research Laboratories (BRL) have a **nonradioactive nucleic acid detection system** called BluGENE that they think will turn the heads of autoradiography users (Reader Service No. 116). Using a streptavidin-alkaline phosphatase conjugate developed at BRL, the BluGENE method can detect single-copy genes of genomic DNA in Southern blots in less than one hour. BluGENE's detection levels reach down to the 0.25 pg level. BRL says the system has several advantages over autoradiography, and by circumventing sandwich methods, their conjugate heightens sensitivity and saves time. Biotin-labelled probes, once prepared, are good for at least a year, and signal development is complete in three hours. BluGENE also avoids the risk, expense and complications surrounding

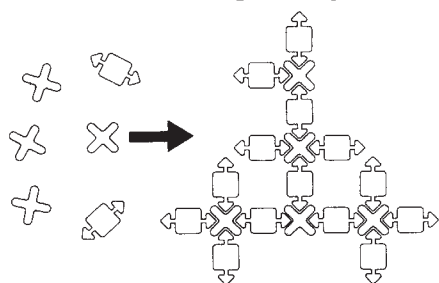


A novel streptavidin conjugate from BRL.

radioactive labelling techniques (as do all nonradioactive methods). BRL prices their BluGENE kit at \$75 (US) for 40 assays; they are in booths D31 and D33 at ASBC.

Photo-activatable biotin (PAB) from Clontech has many of the same advantages as BRL's system (Reader Service No. 117). Determined colorimetrically, PAB's detection can be as sensitive as that afforded by ^{32}P , according to Clontech. One μg of PAB will label 1 μg of DNA, and the label sells for \$75 (US) per mg or \$300 (US) for 5 mg.

Streptavidin, a protein product of *Streptomyces avidinii*, is counterpart to biotin in nonradioactive signal amplification

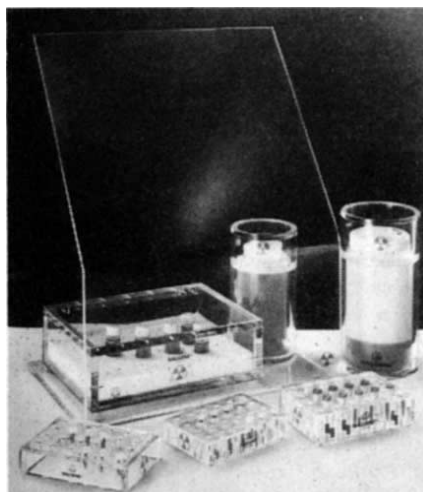


Signal amplification via streptavidin-biotin complexes.

systems. Porton Products Ltd now makes **streptavidin**, purified to X-ray crystallographic standard, for immunological and biochemical techniques (Reader Service No. 118). In comparison to avidin, streptavidin seems to have numerous advantages, including a reduction in nonspecific background effects and improved performance at neutral pH. The chemical is available in bulk quantities from Porton for £5 (UK) per 100 mg, the minimum order.

Vector Laboratories have their own version of a **biotin labelling reagent**, called Photoprobe (Reader Service No. 119). With photoactivation, Photoprobe biotin can produce stable hybridization probes that can be detected via a variety of enzymatic or fluorescent detection systems (including avidin-biotin interaction). In addition to this new probe, information and protocols for Southern, Western and Northern blot detection using Vectastain ABC and Vectastain ABC-alkaline phosphatase kits will be at Vector's booth E0 at ASBC. These kits provide a means of localizing hybridized biotinylated nucleic acid probes, biotinylated antibodies and biotinylated lectins. Photoprobe sells for \$120 a milligram, while the kits cost \$100 (US) each.

When nonradioactive probe labels can't do the trick, Nalge has **acrylic beta radiation shields** to keep exposure to a minimum (Reader Service No. 120). Although acrylic cannot supply protection against

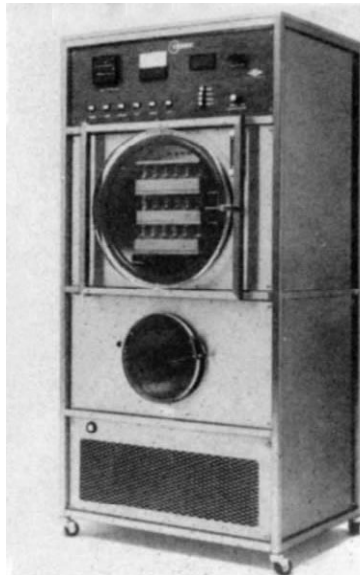


Acrylic shields keep radiation in its place.

gamma radiation or secondary X-rays, Nalge's 3/8-inch-thick layer has been proven effective against the beta emissions from isotopes such as ^{32}P , ^3H , ^{14}C and ^{35}S . Nalge's line includes benchtop shields and waste containers, test tube racks and microcentrifuge tube racks. A standard benchtop shield costs about \$100 (US).

Freeze-dried and frozen

A dedicated **control system for freeze drying** was recently granted a patent, and the company holding the patent will be at ASBC showing their wares. VirTis Co., in booths H27 and H29, devised the FCP-II microprocessor-based system with electronic circuitry and a three-way mixing



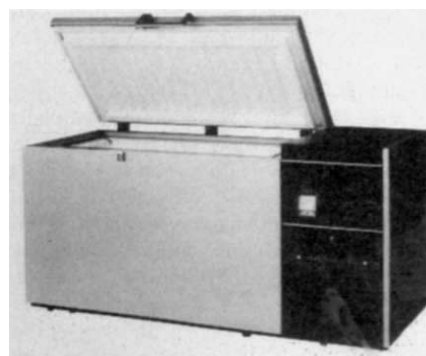
VirTis has freeze drying under control.

valve that can ensure shelf temperature control to $1/10^\circ\text{C}$ (Reader Service No. 121). Their programmer requires no special computer training, has complete manual override capability and provides a hard copy summary (in keeping with FDA requirements) at the end of each run. Any combination of heating, cooling and vacuum level control can be pre-programmed and used repeatedly from

memory. The FCP-II takes readings from 14 different probes monitoring product, shelf and condensor; if any sensors become defective, it discounts their readings, and it automatically takes corrective measures if part of the system malfunctions. VirTis's \$29,750 (US) software and hardware fits all VirTis SRC sublimators.

Fisher just published a 16-page colour brochure detailing their new Isotemp family of laboratory cold units (Reader Service No. 122). *Fisher Isotemp Refrigerators, Freezers, Ultracold Freezers* describes several acid-and-chemical resistant general-purpose refrigerators, chromatography refrigerators, ultra-cold freezers for research and red cell storage and units approved for whole blood and plasma storage. Also making an appearance, above and beyond the 23 models introduced in the booklet, is a variety of new accessories — and plenty of penguins.

The Supercold 135, a recent addition to Gallenkamp's **freezer** collection, runs

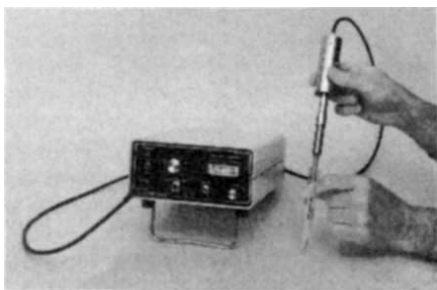


The deathknell for LN₂ storage?

from a standard wall socket and may jeopardize the popularity of conventional LN₂ storage devices (Reader Service No. 123). The unit is part of a generation of very low temperature freezers posed as an alternative to LN₂ freezing; it combines digital temperature display with a convenient height (37 inches) and a lid that makes opening and closing less of a struggle. For about £10,000 (UK) and potentially less power consumption, the Supercold 135 might yet beat out liquid nitrogen varieties.

Cells in pieces

An **ultrasonic cell disrupter** that can homogenize, disaggregate, disintegrate and otherwise influence sample integrity will be demonstrated at booth G42, Sonics & Materials' display. The 40 W model VC-40 is compact and portable, designed for processing small laboratory samples from 100 μl to 25 ml (Reader Service No. 124). The functions of S & M's machine are multi-faceted: it can disrupt cells, bacteria, spores or tissue; prepare an emulsion down to $1/100\ \mu\text{m}$; homogenize liquids; disaggregate particulates; solubilize difficult compounds; and prepare



Portable perturbation for intact cells.

samples for electron microscopy and particle size analysis. S & M says their instrument will also accelerate and catalyse chemical reactions and degas liquids, as well as extract serums, toxins, enzymes and viruses from organic sources. For \$1,080 (US), the VC-40 covers all bases.

For biochemists studying cell surface reactions, Genetic Research Instrumentation Ltd. has presented a **compact electrofusion instrument** that punches holes in the cell walls of plant and microbial cells, or the cell membranes of animal cells (Reader Service No. 125). These holes, according to GRI, can facilitate the incision of material into cytoplasm or improve the yields of cell fusion experiments; GRI reports up to 50-fold increases in incision for its electroporation systems. The Labpulse electrofusion/poration apparatus consists of an oscilloscope to determine pulse shape and strength and control circuitry for voltage outputs of 1 to 1,000 V, which generates pulses 5 ms to 150 ms and pulse-by-pulse repetition to 10 Hz. The system is fully integrated, and the electrodes, which fit into 9 ml polypropylene tubes, can be sterilized by autoclaving or methanol washing and flaming. GRI's instrument works by creating a voltage gradient across the cells that is carefully controlled to puncture, rather than rupture, the cells. Labpulse sells for £5,500 (UK).

The Artek **dismembrator**, model 301, makes many of the same claims for ultra-

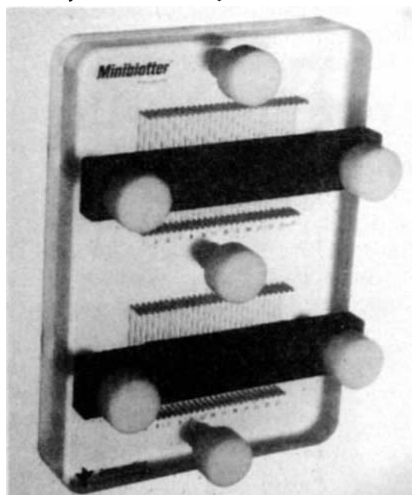


Artek's dismembrator: a disturbing prospect.

sonic disruption as do Sonics & Materials for their instrument (Reader Service No. 126). But Artek's model is not compact; this 2-litre 300 W version features a proprietary feedback system to keep the dismembrator working at maximum efficiency, and the percentage of ultrasonic power emitted is indicated by an output meter. Artek will supply a standard 3/4-inch tip, a 5/32-inch diameter microtip or a 3/8 diameter intermediate tip to accompany the model 301. In addition to disintegrating most cells, bacteria, spores and tissue, Artek's 301 will prepare emulsions down to 1/100 μm , homogenize immiscible liquids, accelerate enzymatic and chemical reactions and stimulate bacterial activity, among other things. Artek offers the model 301 at \$1,950 (US).

Enzymes and antibodies

Western blot screening of hybridomas at early stages is made possible by a device recently released by Immunetics that

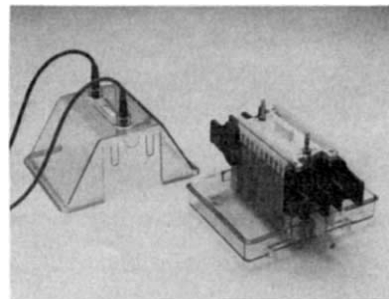


Take the wait out of Western blotting.

required only 100 μl of an antibody solution to perform an analysis (Reader Service No. 127). The Miniblotter, on booth C29 at ASBC, is effective with both hybridomas and serum antibodies: 28 separate antibodies can be screened against a single blot the size of most minigels. Hybridoma supernatants can be transferred directly from 96-well plates into the Miniblotter's channels using a standard 8-channel automatic pipettor. The blot, sandwiched between two plates, receives antibody solutions through holes in the upper plate, and the solutions are kept in their respective channels with a simple clamp system. For larger loads, Immunetics also offer a Miniblotter version with 45 channels, which requires 200 μl of antibody solution. Both sizes cost \$295 (US).

The latest developments at Promega Biotec include an alkaline phosphate-based Western blotting system and ten new restriction enzymes. Promega will

First In Electrophoresis!



Mighty Small II Dual 7cm Slab Unit

Hoefler Scientific pioneered small-gel electrophoresis with its Mighty Small I. The new Mighty Small II can run two 7 x 8 cm gels in as little as 45 minutes—with no smiling. Heat dissipation is outstanding. Gel sandwiches have alumina back plates that conduct heat 40 times faster than glass. Also, there is a serpentine cooling channel sealed between the two vertical upper buffer chambers.

Formerly, Hoefler's Mighty Small I was distributed through Bio-Rad as its Model 360. Hoefler products are no longer available from Bio-Rad. Mighty Small I and II and all parts and accessories are available from the distributors listed below.

- AUSTRALIA** Narellan, NSW
Edwards Instrument (02)605-6777
 - AUSTRIA** Vienna
Aust-Trade (0222) 88 73 13
 - BELGIUM** Brussels
Van der Heyden S.A. 02/21.20.611
 - CANADA** Mississauga, Ont.
Technical Marketing (416) 826-7752
 - FRANCE** Illkirch Cedex
BIOBLOCK 88 67 14 14
& Vanves Cedex 146 44 46 46
 - GERMANY** Heidelberg
Atlanta GmbH 06221/502144
 - JAPAN** Tokyo
Central Scientific (03) 806 4361
 - THE NETHERLANDS** PJ de Meern
Brouwer Scientific 03406/64'299
 - SOUTH AFRICA** Tokai, Cape
Scientific Associates 53 7400
 - SPAIN** Madrid
Iberlabo S.A. 251 14 91
 - SWITZERLAND** Lucerne
Brouwer Engineering 041/44.44.48
 - TAIWAN** Taipei
Mightek Scientific (02) 721-5482/3
 - UNITED KINGDOM** Luton/Beds
Biotech Instruments 0582 502388
- Reader Service No.4

Hoefler Scientific Instruments

P.O. Box 77387, San Francisco, CA 94107
TELEX: 477078

have plenty of information on both of the fledgling products at their ASBC booths GO1 and GO2. The \$175 (US) ProtoBlot immunoblotting system is a nonradioactive method which Promega claims has greater sensitivity than either protein A or HRP-based systems (Reader Service No. 128). The restriction enzymes come with enzyme-specific 10× digest buffer and acetylated nuclease-free bovine serum albumin, both of which have been optimized for reaction efficiency (Reader Service No. 129).

A recombinant protein A for the study of cell surface antigens and receptors and for the detection of antibody-secreting cells has emerged from the laboratories at ICN Immuno-Biologicals (previously ICN Biomedicals). The high-affinity protein is just one of the many chemicals for immunology research that ICN will feature at their booths J15 through J19 (odd) (Reader Service No. 130). Other recent introductions, such as monoclonal and polyclonal antibodies for cytoskeletal elements, should be prominent in ICN's display.

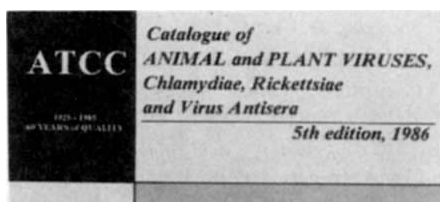
A neurotransmitter commonly known as AMPA (and less often called 3-isoxazolol amino acid (RS) α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid) is a newcomer to Cambridge Research Biochemicals' cadre of neurotransmitters. AMPA, a synthetic analogue of the less stable natural compound ibotenic acid, is a powerful agonist of a subclass of glutamic acid receptors (Reader Service No. 131).

From the pamphlet file

"Filtration — the separation of materials from a gas or a liquid — is one of the most basic and important laboratory procedures." Thus begins Fisher Scientific's new catalogue, the *Fisher Filtration Catalog* (Reader Service No. 132). In full colour spreads, the brochure follows products for micro, macro and process filtration down avenues of applications in biological, microbiological, clinical and pharmaceutical procedures.

As Bond Elut and Chem Elu sorbent extraction methods gain a healthy reputation, keeping track of their potential uses becomes more difficult. Analytichem has published a bibliography listing nearly 500 applications for their two extraction methods as a resource for method development ideas (Reader Service No. 133).

The fifth edition of the American Type Culture Collection's *Catalogue of Animal and Plant Viruses* has now made it to the scientific newstands (Reader Service No. 134). This year's 193-page brochure houses descriptions of over 2,200 strains,



The consummate bug collection.

including molecularly cloned viruses. At ASBC, information on how to obtain a free edition can be had at ATCC's booth C32.

Late in March of this year, BioSciences Information Service (BIOSIS) launched a free sample issue campaign and a discount

programme for its *BioResearch Today* publication series (Reader Service No. 135). It may not be too late to catch up with these offers at BIOSIS's ASBC booth A27. BIOSIS describes *BioResearch Today* (BRT) as a 'group of monthly current awareness abstract journals' that covers 14 specific research topics such as addiction, carcinogenesis, human and animal aging and pesticides. Each issue offers between 75 and 250 abstracts selected from the latest two issues of *Biological Abstracts*, BIOSIS's main publication. □

These notes are compiled by Karen Wright from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal.

ADVERTISEMENT

JOURNALS FROM MACMILLAN

SCIENTIFIC AND MEDICAL DIVISION

In 1986 Macmillan are launching two new journals for new fast developing areas of science as well as strongly supporting their established titles. The publication of **Anti-Cancer Drug Design** and **Bone Marrow Transplantation** respond to new developments in scientific research, and will provide a valuable forum for exchange of ideas.

At the same time our already established journals — Postgraduate Medical Journal will shortly be celebrating its 60th anniversary — continue to chart and report new developments. If you are out of touch with our journals — or would like to see sample copies of our new ones as they are published, we would be pleased to send you a sample copy.

ANTI-CANCER DRUG DESIGN

NEW JOURNAL — FIRST ISSUE DUE SOON

Bi-monthly

Editors: Dr S. Neidle, Dr S. T. Crooke

The development of new clinically-useful anti-cancer agents is increasingly dependent on rational scientific principles drawn from a wide range of disciplines. This new journal aims to promote such approaches, providing a common meeting ground for medicinal chemists, pharmacologists, biochemists, biophysicists and oncologists involved in both fundamental and clinically oriented aspects.

This journal is published with the support of The Cancer Research Campaign.

£45 (UK) \$95 (USA/Canada) £63 (ROW)

BONE MARROW TRANSPLANTATION

NEW JOURNAL — FIRST ISSUE DUE EARLY 1986

Quarterly

Editor: Dr J. M. Goldsman, Co-Editor: Dr R. P. Gale

A new journal which will publish clinical results in man and experimental results with animals; allografting and autografting; HLA and other methods of selecting donors; graft-versus-host disease and all types of methods to prevent and treat it; and much more.

BRITISH JOURNAL OF CANCER

Monthly

Chairman: Professor D. G. Harnden, Editor: Dr M. Moore

One of the leading cancer journals in the world publishing articles on all the major scientific disciplines. Publishes papers from scientists and researchers throughout the world and the proceedings of meetings of the British Association for Cancer Research and other symposia.

£125 (UK) \$275 (USA/Canada) £165 (ROW)

PHARMACEUTICAL MEDICINE

Quarterly

Editor: Dr R. N. Smith, Assistant Editor: P. J. Keen

... "a quarterly journal, which includes papers on the validation of new and established methods of testing drugs in man written by investigators both inside and outside the pharmaceutical industry. It also provides a forum for the exchange of ideas about issues affecting drug development. These new developments merit support from all involved in the management, sale and use of drugs." — *Lancet*

£48 (UK) \$99 (USA/Canada) £68 (ROW)

BRITISH JOURNAL OF PHARMACOLOGY

Monthly

Chairman: Professor J. F. Mitchell

Secretary to the Editorial Board: Dr G. M. Lees

One of the top five most cited pharmacology journals in the world.

A vital source of relevant information for physiologists, immunologists, molecular biologists.

£175 (UK) \$410 (USA/Canada) £199 (ROW)

POSTGRADUATE MEDICAL JOURNAL

Monthly

Editor: Dr B. I. Hoffbrand

A monthly compendium of current clinical research and practice invaluable to the graduate in training.

£68 (UK) \$155 (USA/Canada) \$85 (ROW)

HUMAN TOXICOLOGY

Bi-monthly

Secretary to the Editorial Board: Professor P. Turner

Covers all aspects of human toxicology embracing both animal research and studies with humans.

£80 (UK) \$168 (USA/Canada) £120 (ROW)

To receive sample copies, details of rates and how to submit papers indicate the titles you are interested in and contact: Alison Baverstock, Macmillan Press, Houndmills, Basingstoke, Hampshire RG21 2XS. Customers in the United Kingdom may mark their envelope FREEPOST and need not affix a stamp.

*Macmillan Press
Scientific & Medical*

Mathematics

Discrete Mathematics. By NORMAN L. BIGGS. *Oxford University Press*:1985. Pp.480. Hbk ISBN 0-19-853252-0; pbk ISBN 0-19-853266-0. Hbk £30.00; pbk £15.00.

Finite Algorithms in Optimization and Data Analysis. By M.R. OSBORNE. *Wiley*:1985. Pp.383. Hbk ISBN 0-471-90539-9. £32.00.

From Error-Correcting Codes Through Sphere Packings to Simple Groups. By THOMAS M. THOMPSON. *The Mathematical Association of America*:1985. Pp.228. Hbk ISBN 0-88385-023-0. £22.

Hamilton's Principle in Continuum Mechanics. By A. BEDFORD. *Research Notes in Mathematics*, 139. *Pitman Publishing Ltd*:1985. Pp.106. Pbk ISBN 0-273-08730-4. £12.50.

Introduction to Mathematical Control Theory, 2nd Edn. *Oxford Applied Mathematics and Computing Science Series.* By S. BARNETT and R.G. CAMERON. *Oxford University Press*:1985. Pp.404. Hbk ISBN 0-19-859640-5. £25.00.

Mathematical Models and Applied Mechanics. By A.B. TAYLOR. *Oxford University Press*:1986. Pp.280. Hbk ISBN 0-19-853533-3; pbk ISBN 0-19-853541-4. Hbk £25; pbk £12.50.

Numerical Analysis of Parametrized Nonlinear Equations. By WERNER C. RHEINBOLDT. *Wiley*:1986. Pp.299. Pbk ISBN 0-471-88814-1. £35.75.

Numerical Solution of Partial Differential Equations: Finite Difference Methods, 3rd Edn. By G.D. SMITH. *Oxford University Press*:1985. Pp.337. Hbk ISBN 0-19-859641-3; pbk ISBN 0-19-859650-2. Hbk £20; pbk £10.95.

Prime Numbers. By WILLIAM and FERN ELLISON. *Wiley*:1985. Pp.416. ISBN 0-471-82653-7. Pbk £51.

Statistical Analysis of Measurement Errors. By JOHN L. JAECH. *Wiley*:1985. Pp.293. ISBN 0-471-82731-2. £35.75.

Astronomy

Armchair Astronomy. PATRICK MOORE. *Norton*:1986. Pp.185. Hbk ISBN 0-393-02253-6. \$16.95.

The Astronomer's Software Handbook. By R.A. MACKENZIE. *Sigma Press*, 5 Alton Road, Wilmslow, Cheshire, UK:1985. Pp.292. Pbk ISBN 1-85058-024-3. £8.95.

The Cambridge Astronomy Guide: A Practical Introduction to Astronomy. By BILL LILLER and BEN MAYER. *Cambridge University Press*:1985. Pp.176. ISBN 0-521-25778-6. £13.95. \$24.95.

Comet! The Story Behind Halley's Comet. By GREG WALZ-CHOJNACKI. *Astro Media*:1985. Pp.64. ISBN 0-918831-51-2. \$9.75.

Comets, Meteors & Asteroids: How They Affect Earth. By STAN GIBILISCO. *Wiley*:1985. Pp.208. Pbk ISBN 0-8306-1905-4. Pbk \$12.95.

Cosmic Rays in Interplanetary Magnetic Fields. By I.N. TOPTYGIN. *D. Reidel*:1985. Pp.375. Hbk ISBN 90-277-1863-6. NP.

Data Analysis in Astronomy. V. DI GESU *et al.* (eds). *Plenum*:1985. Pp.541. ISBN 0-306-42018-X. NP.

Invitation to Astronomy. By SIMON and JACQUELINE MITTON. *Blackwell*:1985. Pp.210. Hbk ISBN 0-631-14699-7. £14.50.

Supernovae: Their Properties and Remnants. G. SRINIVASAN and V. RADHAKRISHNAN (eds). *Indian Academy of Sciences*:1985. Pp.178. NP.

1986 Year Book of Astronomy. PATRICK MOORE (ed.). *Norton*:1986. Pp.231. Hbk ISBN 0-393-30254-7. \$15.95.

Chemistry

Advances in Protein Chemistry, Vol.37. C.B. ANFINSEN, JOHN T. EDSALL and FREDERIC M. RICHARDS (eds). *Academic*:1985. Pp.373. Hbk ISBN 0-12-034237-5. £43, \$49.

Asymmetric Catalysis. B. BOSNICH (ed.). *Martinus Nijhoff*:1986. Pp.160. Hbk ISBN 90-247-3259-X. Dfl. 115, £31.95, \$45.50.

Chemical Demonstrations: A Sourcebook for Teachers. By LEE R. SUMMERLIN and JAMES L. EALY Jr. *American Chemical Society*:1985. Pp.190. Pbk ISBN 0-8412-0923-5. Pbk £19.95.

Chemistry of Coal Conversion. RICHARD H. SCHLOSBERG (ed.). *Plenum*:1985. Pp.336. Hbk ISBN 0-306-41974-2. \$52.50.

Comparison of Ab Initio Quantum Chemistry with Experiment for Small Molecules: The State of the Art. R.J. BARTLETT (ed.). *D. Reidel*:1985. Pp.513. Hbk ISBN 90-277-2129-7. Dfl. 210, £58.25, \$79.

Crystal Structure Analysis. A Primer, 2nd Edition. By JENNY PICKWORTH GLUSKER and KENNETH N. TRUEBLOOD. *Oxford University Press*:1985. Pp.269. Hbk ISBN 0-19-503531-3; pbk ISBN 0-19-503543-7. Hbk £29.00; pbk £17.00.

Crystallographic Computing 3: Data Collection, Structure Determination, Proteins and Databases. G.M. SHELDRICK, C. KRÜGER and R. GODDARD. *Oxford University Press*:1985. Pp.314. Hbk ISBN 0-19-855211-4. £25.00.

Environmental Chemistry. By PETER O'NEILL. *George Allen & Unwin*:1985. Pp.232. Hbk ISBN 0-04-551085-7; pbk ISBN 0-04-551086-5. Hbk £8.95, \$13.95; pbk £18.00, \$25.00.

Ferrohydrodynamics. By R.E. ROSENSWEIG. *Cambridge University Press*:1985. Pp.344. Hbk ISBN 0-521-25624-0. £45, \$69.50.

Grosse Moleküle. Plaudereien über Synthetische und Natürliche Polymere. By H.-G. ELIAS. *Springer Verlag*:1985. Pp.204. Pbk ISBN 3-540-15599-6. DM 29.80.

Hydrogenation Methods. By PAUL N. RYLANDER. *Academic*:1985. Pp.193. Hbk ISBN 0-12-605365-0. £40, \$48.

International Tables for Crystallography. THEO HAHN (ed.). *Reidel*:1985. Pp.119. Pbk ISBN 90-277-1964-0. Pbk Dfl. 27.50, \$8.50.

Technology

Detectors and Chromatography. *Australian Scientific Industry Association. Proceedings of an International Conference, May 30 to June 3, 1983.* A.J.C. NICHOLSON (ed.). *Australian Scientific Industry Association*:1986. Pp.198. Pbk ISBN 0-949761-08-7. NP.

Industrial Adhesion Problems. D.M. BREWIS and D. BRIGGS (eds). *Orbital Press/Wiley*:1985. Pp.298. ISBN 0-946193-01-0. £40.

Industrial Hygiene Aspects of Plant Operations. Vol. 2 Unit Operations and Product Fabrication. LEWIS J. CRALLEY, LESTER V. CRALLEY and JOHN E. MUTCHLER (eds). *Macmillan*:1985. Pp.537. ISBN 0-02-949360-9. £65.

An Introduction to Scanning Acoustic Microscopy. By ANDREW BRIGGS. *Oxford University Press*:1985. Pp.70. Pbk ISBN 0-19-856412-0. £5.95.

Liquid Phase Sintering. By RANDALL M. GERMAN. *Plenum*:1985. Pp.240. Hbk ISBN 0-306-42215-8. NP.

Snack Food Technology, 2nd Edn. By SAMUEL A. MATZ. *AVI Reidel*:1985. Pp.415. ISBN 0-87055-460-3. Dfl. 180, £49.95.

Statics and Applied Strength of Materials. By RAYMOND F. NEATHERY. *Wiley*:1985. Pp.655. Hbk ISBN 0-471-88811-7. £35.75.

Techniques for the Automated Optimization of HPLC Separations. By JOHN C. BERRIDGE. *Wiley*:1985. Pp.203. ISBN 0-471-90861-4. £22.

Teleoperation and Robotics: Applications and Technology. Robot Technology, Vol. 3B. By JEAN VERTUT and PHILIPPE COIFFET. *Kogan Page, London, UK*:1985. Pp.256. Hbk ISBN 0-85038-962-3. £32.50.

Computer Science

Compulsive Technology: Computers as Culture. TONY SOLOMONIDES and LES LEVIDOW (eds). *Free Association Books, London, UK*:1985. Pp.160. Pbk ISBN 0-946960-20-0. NP.

Empirical Foundations of Information and Software Science. JAGDISH C. AGRAWAL and PRANAS ZUNDE (eds). *Plenum*:1985. Pp.415. Hbk ISBN 0-306-42091-0. NP.

The Principles of Computer Hardware. By ALAN CLEMENTS. *Oxford University Press*:1985. Pp.454. ISBN 0-19-853704-2. Hbk £25, pbk £12.50.

Selective Computation. By RICHARD E. BELL-MAN. *Wiley*:1985. ISBN 9971-966-86-7. £23.85.

Supercomputers in Theoretical and Experimental Science. JOZEF T. DEVREESE and PIET VAN CAMP (eds). *Plenum*:1985. Pp.225. Hbk ISBN 0-306-42107-0. NP.

Earth Sciences

Advances in Soil Science, Vols 2 & 3. B.A. STEWART (ed.). *Springer-Verlag*:1985. Vol. 2 pp.235. ISBN 0-387-96114-3. DM 148. Vol. 3 pp.217. ISBN 0-387-96116-X. DM 138.

Aspects of the Analysis of Plate Structures. A Volume in Honour of W.H. Wittrick. D.J. DAWE, R.W. HORSINGTON, A.G. KAMTEKAR and G.H. LITTLE (eds). *Oxford University Press*:1985. Pp.332. Hbk ISBN 0-19-856168-7. £45.00.

Down to Earth: One Hundred and Fifty Years of the British Geological Survey. By H.E. WILSON. *Scottish Academic Press*:1985. Pp.189. ISBN 0-7073-0473-3. Pbk £9.50.

Formation Evaluation: Geological Procedures. The EXLOG Series of Petroleum Geology and Engineering Handbooks. ALUN WHITTAKER (ed.). *D. Reidel*:1985. Pp.183. Hbk ISBN 90-277-1978-0. Dfl. 115, £31.95.

The Future of Geography. R.J. JOHNSTON (ed.). *Methuen*:1985. Pp.342. Hbk ISBN 0-416-36690-2. £19.95.

Gravity Field, Seismicity and Tectonics of the Indian Peninsular and the Himalayas. By R.K. VERMA. *D. Reidel*:1985. Pp.213. Hbk ISBN 90-277-1864-4. £36.23, \$44.00.

Improvement of Desert Ranges in Soviet Central Asia. NINA T. NECHAEVA (ed.). *Horwood Academic Publishers*:1985. Pp.327. Hbk ISBN 3-7186-0222-9. \$130.00.

Issues in Atmospheric and Oceanic Modeling. Part A, Climate Dynamics. SYUKURO MANABE (ed.). *Academic*:1985. Pp.591. Hbk ISBN 0-12-018828-7. £79, \$89.50.

General

Problems in Combinatorics and Graph Theory. By IOAN TOMESCU. *Wiley*:1985. Pp.335. ISBN 0-471-80155-0. £36.95.

A Recapitulation of the Lord's Prayer. By J.P. ROSS. *Element Books*:1985. Pp.148. ISBN 0-9510274-0-9. £4.95.

Research in Technical Communication: A Bibliographic Sourcebook. MICHAEL G. MOKRAN and DEBRA JOURNET (eds). *Greenwood Press*:1985. Pp.515. ISBN 0-313-23431-0. £63.50.

Residential Development and the Planning System: A Study of the Housing Land System at the Local Level. By Y. RYDIN. *Permagon*:1985. Pp.69. ISBN 0-08-032742-7. NP.

The Riemann Zeta-Function: The Theory of the Riemann Zeta-Function with Applications. By ALEXANDER IVIC. *Wiley*:1985. Pp.517. ISBN 0-471-80634-X. £57.80.

Sagamore Army Materials Research Conference Proceedings, Vol. 30: Innovations in Materials Processing. GORDON BRUGGEMAN and VOLKER WEISS (eds). *Plenum*:1985. Pp.519. ISBN 0-306-41839-8. NP.

Scientific Realism. JARRETT LEPLIN (ed.). *University of California Press*:1984. Pp.266. Hbk ISBN 0-520-05155-6; pbk ISBN 0-520-05326-5. Hbk \$32.75, £29.95; pbk \$11.50, £10.50.

Shall We Make the Year 2000? The Decisive Challenge to Western Civilization. By J.G. DE BEUS. *Sidgwick & Jackson*:1985. Pp.183. ISBN 0-283-99221-2. £12.95.

Speech Science. RAYMOND G. DANILOFF (ed.). *Taylor & Francis*:1985. Pp.326. ISBN 0-85066-500-0. £22.

Structure in Thought and Feeling. By SUSAN AYLWIN. *Methuen*:1985. Pp.274. ISBN 0-416-35990-2. £25.

Symmetry and Structure. By S.F.A. KETTLE. *Wiley*:1985. Pp.330. ISBN 0-471-90705. NP.

Temporal Order. L. RENSING and N.I. JAEGER (eds). *Springer-Verlag*:1985. Pp.325. 3-540-15274-1/0387-15274-1. NP.

Urban Geomorphology in Drylands. By R.U. COOKE *et al.* *Oxford University Press*:1985. Pp.324. ISBN 0-19-823258-6. £9.95.